A Study of Glutathione in Health and Certain Disease States

by

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ERRATA

Page 48: Line 4, "Hepatis" to read "hepatitis".

Page 18, "Hb" to read "Hb%".

Page 18, "Dehydroisoandriosterone" to read "Dehydroisoandrosterone".

Page 24, "Prothrobin" to read "Prothrombin".

Appendices x "Mr. M.B." to read "Mrs. M.B."

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INTRODUCTION

For Orientation

This investigation was originally intended to study glutathione in relation to diabetes as a result of the discovery of glutathione protection against alloxan diabetes. It was proposed to study blood levels of glutathione in all types of diabetic patients, in those patients showing the complication of diabetes and in those diseases which tended to make diabetes more severe. When it was realized that glutathione is contained primarily in the erythrocytes and not in the plasma it was felt that a measure of the glutathione content of the erythrocytes was of more value than blood levels of glutathione. It was then discovered that in certain disease states there was an alteration in glutathione content of erythrocytes which will be reported. Since the study was originally intended to study the etiology of diabetes in relation to glutathione, the reader will find many references to diabetes in the discussions of other diseases.

History of Glutathione

Glutathione was isolated by Hopkins (1) in 1921 who showed that it was very widely distributed in living cells. He stated it was a dipeptide containing cysteine and glutamic acid. The following formula was worked out by Quastel, Stewart and Tunnicliffe in 1923.

CH2
CH-----NH-----CO.CH2.CH2.CHNH2.COOH
COOH

Hunter and Eagles (2) in 1927 suggested that glutathione might be a tripeptide and that serine might also be a constituent of the molecule. In 1929 Hopkins (3) reinvestigated his original work and found that glutathione was a tripeptide containing cysteine, glycine, and glutamic acid. He succeeded in finding a method for the ready isolation of glutathione in the pure crystalline state. Kendall, McKenzie and Mason in 1929 (4) confirmed the work of Hopkins that glutathione was a tripeptide and that it was formed from cysteine, glycine and glutamic acid.

HOOC.CH(NH2).CH2.CH2.CO.NH.CH(CH2.SH)CO.NH.CH2.COOH.

Distribution of Glutathione

Glutathione is found almost exclusively in the intracellular fluid. In the blood it is found in the red blood corpuscles and is mostly in the reduced form. Of interest is the high concentration of glutathione in the lens of the eye (5,6), and its marked reduction when a cataract forms (5,7). The spinal fluid, normally being devoid of cells is reported not to contain glutathione (8).

Synthesis of Glutathione

A rapid incorporation of the N15 from labelled glycine and glutamic acid into the glutathione of livers and small intestines of intact animals was demonstrated by Waelsh and Kittenberg (9,10). They showed the half lifetime of glutathione in the livers of rats and rabbits to be about two to four hours. Braunstein et al (11) succeeded in demonstrating the synthesis of glutathione from the three component aminoacids in tissue slices of rat livers. Block et al

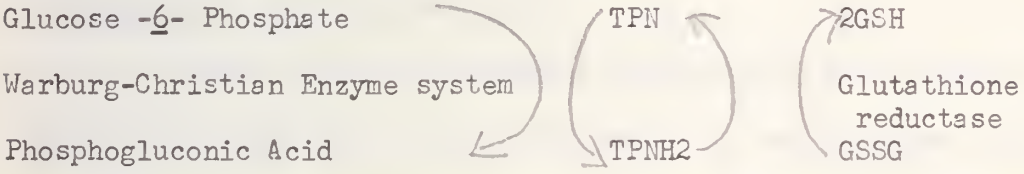
(12,13,14,15)showed that the supernatant solution of pigeon liver homogenates contained the enzyme system responsible for the introduction of labelled glycine into glutathione. They also showed that C14 glycine, N15 glutamic acid and N15 glycine result in the formation of labelled glutathione. It was found also that adenosine triphosphate markedly accelerated the aerobic synthesis of glutathione in liver homogenates. Anderson and Mosher (16) have demonstrated that the intestinal mucosa may also be a site of glutathione synthesis using S35 labelled DL.cystine. The maximum incorporation of cystine sulfur into liver glutathione they estimated to take place in the rat in less than three hours. Linden and Work (17) found that rats given a deficient diet of the sulfur containing aminoacids had reduced (reduced and total) glutathione values. Binkley et al (18) have found that the giving of insulin caused a marked decrease of glutathione in the liver. The glutathione content of blood has been reported to fall in hepatectomized animals and in patients with acute hepatitic disease which suggested that the glutathione of erythrocytes was derived from the liver. However, the possibility has not been excluded, as Binkley states, that the role of the liver might be to furnish the constituent aminoacids for the synthesis of glutathione by erythrocytes and other tissues, or insulin may act as a stimulant for the removal of glutathione from the liver by the erythrocytes. Of interest is the work of Kinsey and Merrian (19), who, using glycine with radioactive C14 were able to show glutathione synthesis in situ in the lens. Binet et al (20) have also shown that when the suprarenal gland is perfused

with cystine and glutamic acid there develops a marked increase in the glutathione content of the perfused gland (200 mgms. over the control gland) and also a marked increase in glutathione content of venous blood over arterial blood.

Metabolism of Glutathione.

Glutathione in the extracellular fluid may be oxidised by the catalytic action of copper (21). Keilin (22) showed that glutathione inside the cells maybe oxidised by the catalytic action of the cytochrome oxidase-cytochrome C system. Ames and Elvejhem (23,24) also found that reduced glutathione could be oxidised by an enzymatic system involving cytochrome C. The glutathione oxidase system is strongly inhibited by heat, cyanide, diethyldithiocarbonate and iodacetate. They found that co-enzyme 1 increased the rate of enzymatic oxidation of reduced glutathione in the absence of cytochrome C and decreased the induction period either in the presence or absence of cytochrome. They found that adenosine triphosphate had no effect on the enzymatic oxidation of reduced glutathione. Ascorbic acid was found to catalytically stimulate the oxidation of reduced glutathione by cell free tissue preparations both in the presence or absence of cytochrome C. Purr (25) showed that glutathione could be oxidized by hydrogen peroxide and peroxidase. Hopkins and Elliott (26) showed that the livers of well fed animals reduced oxidized glutathione rapidly, and the reduced glutathione was also rapidly oxidized and found also that these processes were greatly reduced in the livers of starved animals- and indication that reduction was correlated with the oxidation of metabolites.

Mann (27) showed that glutathione is reduced by glucose in the presence of glucose dehydrogenose prepared from liver. The reaction proceeds slowly, but can be greatly accelerated by an activator which can be extracted from liver. Meldrum and Tarr (28) showed that glutathione is reduced both aerobically and anaerobically by the Warburg --Christian enzyme -- co-enzyme system in the presence of hexosemonophosphoric acid.



TPN is co-enzyme II - The Warburg-Christian co-enzyme is present in red blood corpuscles.

Hydrolysis of Glutathione

Binkely et al have studied the hydrolysis of glutathione very extensively. They (29) believe the hydrolysis of glutathione to cysteine is a two-step process involving the intermediary formation of cysteinylglycine. No y-glutamylcysteine was found. The enzyme responsible for the hydrolysis of glutathione to cysteinylglcine was, in the rat, they found limited to the kidney and intestine. The enzyme responsible for the hydrolysis of cysteinylglcine was found in muscle and liver as well as in kidney, and they felt it probably occured inall tissues. Their results (30) suggested that a physical as well as a functional relationship exists between the glutathionase and cysteinylglcinase of kidney tissue. Cysteinylglycinase has been found to be activated by manganous, cobaltous and ferrous ions. It has been found (31) that glutamine is required for the hyrolysis of glutathione by enzymes of kidney tissues. The site of

action of glutamine was upon the first step -the hydrolysis to cysteinylglycine-. Since glutamine is not hydrolyzed by the system, they have concluded that glutamine was acting as a co-enzyme. Penicillin and bromosulfalein were found to be inhibitors of the hydrolysis. They believe (32,33,34) glutathionase is a lipoprotein and cysteinylglycine maybe a pentose nucleic acid.

Glutathione in Peptide Fermation.

Hanes, Hird and Isherwood (35,36) have shown that glutathione will react with various animoacids in the presence of an enzyme from kidney and other tissues to form new peptides containing glumatic acid. Their evidence shows that the new peptides formed were y-glutamyl peptides and that the reaction consisted of the transfer of the y-glutamyl group from its linkage in glutathione to linkage with the amino group of an "acceptor" aminoacid. They suggest, as also have Fruton et al, (37) that glutathione may participate in enzyme-catalyzed transamidation reactions in protein synthesis. Binkley (32) suggests, because of the limited distribution of glutathionase, that the hydrolysis of glutathione is intimately concerned with absortive processes, and in particular, with the function of the intestine and kidney.

Glutathione and Sulfhydryl Enzymes

Sulfhydryl groups are common to many enzymes, and inhibitors of these enzymes react by combining with the -SH group. e.g. heavy metal, or by causing oxidation of the -SH group. Glyoxalase is the only enzyme system known in which glutathione acts as a co-enzyme. Hopkins and Morgan (38) have shown gloxalase is

widely distributed in living organisms and the activity of the enzyme is limited by the amount of glutathione present in the cells. Barron and Singer (39,40) showed that a great many enzymes concerned with carbohydrate, fat and protein metabolism were -SH enzymes. Kremsky and Racher (41) have shown that glyceraldehyde -3- phosphate dehydrogenase contains a firmly bound prosthetic group which they have identified as glutathione by several independent tests. Roughton and Clark (42) state that glutathione is an activator of carbonic anhydrase. Whenever the thiol groups of these enzymes are attacked by agents which destroy the -SH groups, glutathione will restore the SH groups by withdrawing the heavy metal or by reducing the oxidized -SH groups.

Glutathione as a Regulator of Enzyme Action

Barron et al (43) by measuring the respiration of sea urchin sperm treated with a variety of -SH reagents: oxidizing, alkylating and mercaptide forming agents, found that when these reagents were employed at low concentrations, just enough to combine with, or oxidize the soluble groups present in the cell, there was an increase in cell respiration. As the concentration of the -SH reagents was increased, the fixed -SH groups, those in the side chains of the protein, were attacked, and inhibition of respiration ensued. They (43,44) postulate therefore, that there are, in living cells, two types of thiol groups: the soluble thiol groups, mostly glutathione, which belong to the regulatory mechanisms of cellular metabolisms; and the fixed thiol groups, which are in the side chains of the proteins. Thiol enzymes belong to this latter group.

Glutathione and Ascorbic Acid

Glutathione is known to prevent the oxidation of ascorbic acid (45). Barron states (46) that both substances are universally distributed in living cells, mostly in their reduced state; both exist in greater concentrations in embryonic tissue, where the synthetic processes occur with greater intensity and both are sluggish oxidation-reduction systems, not oxidized in atmospheric oxygen unless in the presence of a catalyst. In the absence of ionic copper in the cells, where copper is mostly bound to aminoacids or protein, glutathione may protect ascorbic acid from oxidation.

Glutathione in Relation to Cell Division and Growth

Rapkine, (47,48,49) twenty years ago, found that glutathione was associated with cell division and he showed that there was a marked increase in the glutathione content of fertilized sea urchin eggs just before cell division. Addition of $HgCl_2$ stopped mitoses completely. Voegtlin and Chalkley (50) showed that glutathione added to ameba proteus caused increased nuclear and cell division and more polynucleated cells, showing that glutathione had more effect on nuclear division than on cell division. Rapkine postulated that acceleration of the processes of cell division and cell growth was due to a decrease in the oxidation-reduction potential in the cell, i.e. an increase in the reduction intensity. He believed that the increased -SH groups were produced by a reversible denaturation of intracellular proteins which transformed masked and sluggish -SH groups into freely reactive -SH groups. Binet et al (51,52) found in plant tumors there was a marked increase

in glutathione content, and that the increase of glutathione is a function of the rapidity of growth of the tumor. Brachet (53) agrees with Rapkine's postulation. Glutathione has been shown to be a nutritional requirement of Hemolytic streptococci (54) and Neisseria gonorrhoea(55). The growth inhibiting action of -SH reagents on cultures of Escherichia coli, Eberthella typhosa and staphylococcus aureus could be combated by the addition of cysteine and glutathione, but not by cystine or methionine (56).

Glutathione and Ionizing Radiations

Glutathione and other thiols have been studied extensively by different investigators in relation to ionizing radiations. Barron(57) postulated, in 1944, that ionizing radiations would rapidly oxidize the thiol groups of cells. Kinsey (58) previously, in 1935, showed that X-rays had a destructive effect on aqueous solutions of glutathione. Barron and Flood (59) found that glutathione was readily oxidized by X-rays, beta rays and gamma rays. Barron et al (60,61) showed that dilute solutions of sulfhydryl enzymes showed reduced activity on irradiation by small amounts of X-rays. When the inhibition was partial, the enzyme was reactivated on addition of glutathione showing that the inhibition was produced by oxidation. Le May (62), studying the succinic oxidase system as well as its component enzymes in rat kidneys following irradiation, found no inhibition, and he questions any selective in vivo effects of moderate doses of irradiation on -SH enzymes. Dale and Davies (63) have shown that apart from any possible reversible oxidations that

may occur, there are also irreversible changes produced by X-rays leading to the liberation of hydrogen sulfide. This has been shown to occur with cysteine and glutathione. Feinstein (64) demonstrated that cysteine and glutathione, if added before irradiation, prevented the reduction in viscosity of alkaline nucleoprotein solutions. Fischer et al (65) found that the reduced glutathione content of blood, liver, heart, kidney or muscle was not lowered following fatal X-ray dosages in mice and guinea pigs. Peterson et al (66) found that in rats total body irradiation of 500 r generally caused a decrease in blood glutathione (mgms,%), associated with a drop in hematocrit, whereas, correcting for the hematocrit there was actually an increase in glutathione in the red blood corpuscles following irradiation. Patt et al (208) showed that cysteine and glutathione intravenously greatly reduced the sensitivity of rats and mice to lethal amounts of X-ray provided it was given before exposure. Chapman and Cronkite et al (67 - 72) found that the survival rates of glutathione treated rats were greater than those not treated, and that these animals lost less weight, recovered weight more rapidly and withstood trauma better than the control animals. They found also that glutathione given before irradiation to mice seemed to protect the mechanisms which accelerate regeneration of the hemopoietic and myelopoietic tissues. They followed the clearance of intravenously given glutathione in dogs and found that the extra glutathione rapidly disappears from blood and that the distribution of this

glutathione is not uniform, but the highest concentrations were found in the liver, spleen and kidneys. They suggest that glutathione protection when given before irradiation, is due to it being concentrated in vital organs, liver, kidney and spleen. Shirai (73) has reported beneficial effects following glutathione treatment of thirty-two patients who were known to be suffering from roentgen intoxication as a result of X-ray therapy for uterine carcinoma. Mikaelson (74) has shown that glutathione prevents the developement of radiation-induced chromosone aberrations. Fulton et al (75) have shown that glutathione appears to protect also the animals immune defensive systems from the effects of X-radiation or enhance them after they have been impaired as a consequence of the X-radiation.

ESTIMATION OF GLUTATHIONE IN BLOOD

There have been many methods developed for the estimation of glutathione (76-83, 85-89).

The method of Binkly et al (80) attempts to measure not only glutathione but also the products of its metabolism which are cysteinylglycine, y-glutamylcysteine and cysteine. Cysteine is not found normally in blood. This method was attempted at first. (see appendix for details of methods as described). Many difficulties were encountered. Sodium-B-naphthaquinone-4-sulfonate which was old stock gave a cloudy solution instead of a clear solution as a new stock later gave. At the same time this reagent should be made up fresh daily, a fact which Binkley did not bring out in his articles. Sodium thiosulfate, also old stock, did not allow the final color reaction to remain stable long enough for reading purposes. When new stocks of these reagents were obtained these difficulties disappeared. Their method (80) was very briefly described, (see appendix), subsequently many interpretations of the different procedures could be found. It was difficult to decide where, in the method of Sullivan and Hess (84) (see appendix) one should start, and after consultation with others, it was decided that the addition of 5% NaCN was not necessary. After a great deal of work without satisfactory results, I then wrote to Binkley, who sent me mimeographed copies of his method. (see appendix) Here many of the steps are described in more detail and amounts and concentrations of reagents changed from that described previously.

Their readings, as reported, were made using a Coleman junior clinical spectrophotometer. This equipment was not available at the University of Alberta Hospital and a Beckman Model DU spectrophotometer was used. A standard solution of 0.5 mgms/cc glutathione with this equipment gave a reading of around 80% light transmission at 540 mu. which was not in the range of greatest accuracy for reading; whereas using their equipment, they obtained readings of 53-55% light transmission for a solution of 0.5 mgms/cc.glutathione. In order to concentrate the final volume to increase the color concentration, the concentration of the reagents of Sullivan and Hess were increased, and the volume of each added, was decreased proportionately so that the final volume was only 12 cc instead of 17 cc. The readings then for 0.5 mgms/cc glutathione were from 41-44% transmission at 540 mu. but the final color reaction was not stable long enough and it was felt at these concentrations the reagents were not stable. Another difficulty experienced was obtaining a constant 500/580 ratio for cysteinylglycine and cysteine (see table 1). For different concentrations of cysteinylglycine (hydrolyzed glutathione) the 500/580 ratio should have been constant.

Mgms. GSH HYdrolyzed to Cysteinylglycine	500/580 Ratio
0.1	2.2
0.25	1.4
0.5	1.2

Table 1 A comparison of the hydrolyzed Glutathione to the 500/580 ratio (Binkley method).

With standard solutions of cysteine hydrochloride the 500/580 ratio varied from 4.25 to 5.1. The relative concentrations of cysteine and cysteinylglycine are read from a graph of the 500/580 ratios. Binkley et al (5) found the 500/580 ratios for cysteine to be 3.4 and for cysteinylglycine to be 1.3 and the composition of a given solution would be found from a graph using these two values. Without constant values for the 500/580 ratio of pure solutions of cysteinylglycine and cysteine it would be impossible to make such a graph without a large percentage error. Their results (5) show errors in cysteinylglycine and y-glutamylcysteine ranging from 25% to almost 50%. For the reasons given above this method was discarded.

The method of Thompson and Watson (82) was then attempted with success. This is a nitroprusside method which was first described by Fujita and Numata in 1939, and later modified by Bruckman and Wertheimer (13) in 1947. Thompson and Watson have also modified the method in order to increase the stability of the reagents, and increase the color intensity. After the production of color the solution was transferred to Helige 510 tubes and read in the Evelyn Photoelectric Colorimeter, using the Freier-Larsen adaptor and a 515 filter. The final color remained stable long enough for one to get an accurate reading. As they suggested a standard solution of glutathione was included in each series of blood estimations. In almost every series one recovery experiment was carried out and the recoveries were always between 90 and 102 %. A series of duplicate determinations on blood gave results within 2 mgm % (see table 2).

	No.1	No.2
1.	26.0	26.0
2.	26.8	27.6
3.	32.0	32.8
4.	32.4	31.6
5.	18.4	19.2
6.	23.2	22.8
7.	21.6	20.4
8.	21.4	22.0
9.	22.2	22.8
10.	26.8	26.4
11.	20.8	21.6
12.	24.4	25.2
13.	26.8	25.8
14.	23.6	24.2
15.	28.8	28.0
16.	36.0	34.8
17.	38.0	37.6

Table 2 Comparison of duplicate specimens of blood-method of Thompson and Watson.

The results with the nitroprusside method are slightly lower than those by iodometric method as shown By Thompson and Watson (82). They state the slowly developing nitroprusside reaction given by acetoacetic acid roughly equivalent to blood levels of 4 mgms/100 ml. gave no measurable color at 30 seconds, although by 90 seconds a signicent degree of color had developed. Kety et al (252) studied total ketones in diabetic acidosis and reported that in severe diabetic acidosis there were 38.5 to 90.6 mgms % with an average of 66.3 mgms % total ketones present. In diabetic coma they reported 47.7 to 132.5 mgms % with an average of 98.4 mgms % total ketones. The effect of acetone to 100 mgms. % was studied and showed no color reaction, however, ethylaceto acetate gave a marked color reaction even at levels of 25 mgms % acetoacetic acid (see table 3). This must definitely be taken into consideration in reporting the results of patients with diabetic acidosis as will be shown later.

Acetoacetic Acid mgms. %	Color Intensity Compared with Glutathione Concentration
100	20.0 mgms. %
80	16.8 mgms. %
50	13.6 mgms. %
25	9.6 mgms. %

Table 3 The color intensity of acetoacetic acid (as ethylacetoacetate) produced in the method of estimation of Glutathione as described by Thompson and Watson.

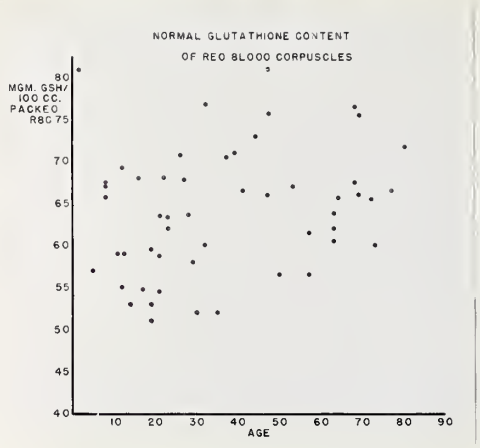

NORMAL GLUTATHIONE CONTENT
OF RED BLOOD CORPUSCLES
MGM. GSH/
100 CC.
PACKED
RBC
80
75
70
65
60
55
50
45
40
10
20
30
40
50
60
70
80
90
AGE

Figure I

NORMAL LEVELS OF GLUTATHIONE IN BLOOD

Most investigators have expressed glutathione values in mgms. %. It soon occurred to us, that, since the glutathione is contained almost exclusively in the intracellular space, a correction should be made for differences in the concentration of erythrocytes in the blood (hematocrit). All glutathione values in this investigation are, therefore, expressed as mgm. glutathione/100 cc. packed erythrocytes. We are therefore showing the comparative amount of glutathione in each erythrocyte, and can compare the amount present even where there is a difference in the hematocrit.

Normal values of glutathione were detemmined from fifty one people ranging in age from eighteen months to eighty years (see figure 1 and appendix). Most of the patients taken as normal were either young people who had had poliomyelitis, but, who were past the acute stage by three or four months, and who were in hospital for treatment, or older people in hospital with fractures. They were all seen and checked for any other illnesses they might have. Results expressed as mgm. glutathione/100 cc. erythrocytes ranged from 51 mgms. to 81 mgm. The average age was 37.4 years and the average glutathione value was 64.0 mgms./100 cc. erythrocytes. As it may be seen from figure 1, there is a suggested increase in glutathione content of erythrocytes with increasing age, which can be shown statisically (see table 4). The value of t is 2.291 with 48 degrees of freedom. The 5% value is 2.01. The probability of deviation from 0 as great as that found was 0.04 which is significant.

This slope is significantly different from 0 at the 5% level.

Age	y= a + b x	Mgm. GSH/100 cc. R.B.C.'S
5	60.17 + 0.1024 X5	60.68 mgms.
20	60.17 + 0.1024 X20	62.22 mgms.
40	60.17 + 0.1024 X40	64.27 mgms.
60	60.17 + 0.01024 X60	66.31 mgms.
80	60.17 + 0.1024 X80	68.36 mgms.

Table 4 Statistical Values of the Regression Line of mgm. Glutathione/100 cc. packed erythrocytes against age in Normal Indivisuals where y is glutathione content and X is age in years (a = 60.17)

GLOTATHIONE IN BLOOD DISEASES AND IN CIRCUMSTANCES OF POOR BLOOD OXYGENATION

(1) Blood Glutathione in Patients with Anemia.

Glutathione has been studied extensively in relation to blood diseases. Since glutathione is contained in the blood elements and not in the plasma, it is necessary when considering blood levels to correct for the hematocrit reading. Gabbe (90) in 1929 showed in anemia the reduced glutathione content of red blood cells was greater than normal. He showed that in animals the removal of blood caused a reduction of blood glutathione, whereas the amount in each corpuscle went up. He suggested that the purpose of glutathione was not so much for the metabolic needs of the cells, but for assisting in the oxidation and reduction of hemoglobin. Varela et al (92) in 1930 reported low blood glutathione levels in hypochromic and pernicious anemias, but they did not correct for the hematocrit. Since then many investigators (93 - 100) have studied glutathione in relation to anemias and the differences in their findings are mostly due to the fact that some corrected for differences in hematocrit and others did not. Unless corrected for hematocrit the glutathione levels in blood were found to be normal or low whereas if corrected for hematocrit the glutathione content of the red blood corpuscles was found to be above normal. Bickel (94) has shown that hemorrhages in rabbits and man cause a fall in glutathione corresponding to the fall in erythrocytes, but afterwards glutathione values rise more rapidly than the erythrocytes, so that

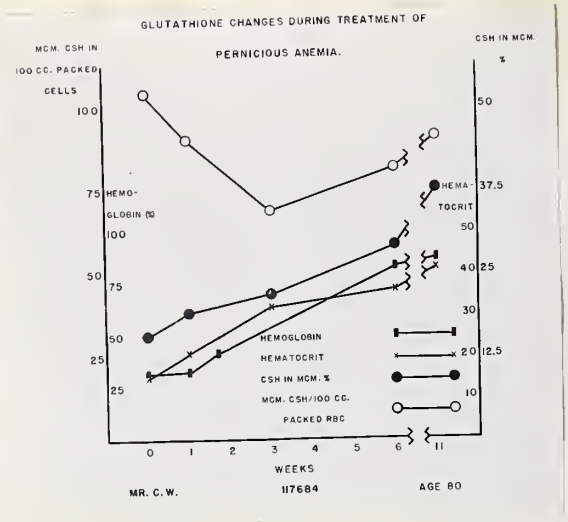


Figure 2

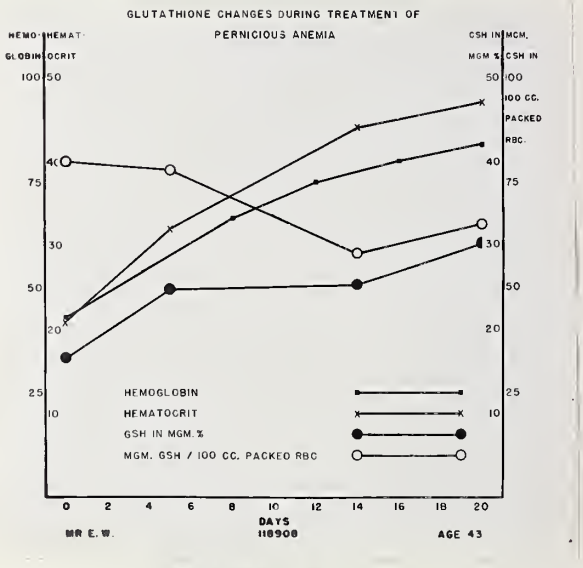


Figure 3

Case No.	Name	Age	Hg.	GSH/mgms.%	Hem.	Mgm.GSH/100 cc. RBC	Comments & Diagnosis
1.	Mrs. H.C.	75	59%	28.8	32	80	Diabetes and Uremia
2.	Mrs. H.K.	63	61% (8.8)	30.2	34	95	Diabetes and Uremia
3.	Mr. O.L	64	57% (8.3)	19.2	28	69	Gastric Ulcer with Haemorrhage
4.	Mr. R.L.	64	47% (6.8)	17.8	27	66	Retroperitoneal Sarcoma Pyrexia
5.	Mrs. B.	80	40%	18.6	20	93	Primary Essential Myeloid Metaplasia.
6.	Mrs. I.F.	47	42% (6gm.)	38.8	30	129	¾ Gastrectomy 5 years ago. Improved on Iron.
7.	Mrs. V.A	72	48%	17.8	30	59	Idiopathic Hypocalceima
8.	Mrs. G.	62	56% (8.2)	19.8	30	66	Multiple Myeloma - Pyrexia
9.	Mr. F.E.	80	52% (7.6)	26.4	30	88	Associated diabetes mellitis
10.	Mr. R.C.	68	59% (8.6)	32.4	35	93	Hypertensive Heart Disease with failure
11.	Mrs. L.H.	71	62% (9.0)	32.8	33	99	Multiple Myeloma, Pyrexia
12.	Mrs. F.K.	44	40% (5.8)	37.8	35	108	Hypochromic Anemia
13.	Mrs. D.L.McD	43	52%	33.2	32	104	Hodgkin's Disease
14.	Mr. A.K.	41	50% (7.2)	45.2	23	197	Bone Marrow Normal
15.	Mr. L.P.	39	40% (5.8)	40.0	31	129	Rheumatoid Arthritis
16.	Mr. C.W.	80	32%	15.6	15	104	Pernicious Anemia
17.	Mr. E.W.	43	43%	16.6	21	80	Pernicious Anemia
18.	Mr. T.M.	53	59%	21.2	33	64	Untreated Diabetes
19.	Mrs. S.J.	28	69%	25.6	30	80	Hypothyroidism and Diabetes
20.	Mr. E.D.	39	62% (9)	32.8	30	109	Hypothyroidism
21.	Mrs. O.	60	73	34	36	95	Probably Hypopituitarism
22.	Mr. A.K.	41	68% (9.8)	37.6	31	121	Chronic Lymphatic Leukaemia
23.	Mr. C.S.	61	62% (9)	22.2	32	74	Possibly Multiple Myeloma
24.	Mr. P.O.	84	51% (7.4)	29.8	29	103	Prostatic Ca. with Metastasis
25.	Mr. I.S.	40	66%	34.0	38	90	Diabetes untreated. Hypertension
26.	Mr. F.H.	63	31% (5.1gms.)	17.2	17	101	Pernicious Anemia.

Table 5 - Glutathione Content of Erythrocytes in Anemia.

Mr. F.H.
Age 63.

Date	Hb.%	GSH mgm.%	Hematocrit	Mgm. GSH/100cc. RBC
21.4.53	31%	17.2	18	101.2
30.4.53	57%	20.4	26	79
7.5.53	69%	17.2	36	46
14.5.53	79%	21.8	39	56

Table 6 - Changes in Glutathione Content Occuring during treatment of Pernicious Anemia with Vitamin B 12.

Name	Date	Amount of blood received	Hb.%	GSH in mgms.%	Hem.	Mgm.GSH/100cc. Packed RBC
Mrs. H.K.	27.1.53	2000 cc. in	61%	30.2	34	95
	5.2.53	4 days	84%	29.8	40	75
Mrs. D.L. McD.	27.1.53	1500 cc.	64%	33.2	32	104
	13.2.53		100%	30.4	48	63

Table 7 - Changes in Glutathione Content Occuring during treatment of anemia with blood transfusions.

the"glutathione index" is increased. In four patients with pernicious anemia he found the "glutathione index" to be very high, being highest where the number of erythrocytes the lowest. As the erythrocytes increased with treatment the blood glutathione (in mgms.%) increased also, but slower than the erythrocyte count so that the "index" decreased.

In this study there were twenty-six patients with anemia (see table 5). The range of glutathione values obtained was from 59 - 197 mgms/100 cc. red blood corpuscles, with an average of 96 mgms/100 cc. red blood corpuscles. Three cases of pernicious anemia were followed while receiving vitamin B12 (see figure 2 and 3 and table 6). It will be noted that as the hematocrit and hemoglobin rose in each case the glutathione content of the red blood corpuscles fell for about the first two weeks, and then began a slow rise. However, in the case of Mr.C.W. (figure 2), even after eleven weeks, when he had returned to active work, the glutathione content of the red blood corpuscles had not returned to its original high level. It might be that the increased levels, after about two weeks, could be correlated with increased activity as the patients improved. In two cases of anemia associated with other conditions (see table 7) there was a marked drop in the glutathione content of red blood corpuscles following elevation of the hematocrit and hemoglobin by means of blood transfusions.

(2) Glutathione Content of Erythrocytes in Patients with Pulmonary Disease.

Condition associated with poor oxygenation of the blood have

been studied in relation to glutathione, Gurevich (101) stated that in pulmonary and cardiac disorders the changes in reduced or total glutathione were not specific for any given disorder. Klotz (102) found that the glutathione content of red blood corpuscles was increased in congestive heart failure, and returned to within normal limits on digitalis therapy. Aisenberg and Khatskevich (103) reported that during the first and second stages of decompensation in congestive heart failure the glutathione content of red blood corpuscles rose. Blyakher (104) also reported high glutathione levels in decompensated heart disease. Rabboni and Buttitta (105) found in animals, following lobectomy and pneumonectomy, an increase in blood glutathione with only a slight increase in hematocrit. Kosyakov (106) and Hosoi (107) found increased glutathione content of erythrocytes during anoxemia and asphyxia respectively. Chervyakovski and Bruk (108) stated that in any condition leading to severe cyanosis, the glutathione content of blood is elevated. Increased glutathione content of erythrocytes at high altitudes have been reported by Paskaeu (109) and others (110,111,112). Vacca (113) also found high glutathione content of erythrocytes at lowered barometric pressures and believed this due to more newly formed erythrocytes in the circulating blood, which he thought might be richer in glutathione than the older erythrocytes. High glutathione levels have been reported in carbon monoxide poisoning by Omura and Ohida (114) and by Yoshizawa (115).

In this study there were ten cases of pulmonary disease

Case No.	Name	Age	Hg.	GSH in mgms.%	Hem.	Mgm.GSH/100 cc. RBC	Commentsand Diagnosis
1.	Mr. J.E.	68	89% (12.9)	49.2	48	103	Bronchogenic Ca. with Cyanosis
2.	Mr. A.J.D.	56	83%	27.6	36	77	Pulm. Fibrosis with Emphysema, Multiple Myeloma.
3.	Mr. E.N.	67	106% (15.5)	44	50	88	Marked emphysema with congestive heart failure.
4.	Mr. J.F.	66	86%	35.2	40	88	Marked emphysema with dyspnoea.
5.	Mrs.S.W.	72	107% (15.6)	52	54	96	Bronchiectosis, emphysema, cyanosis and heart failure.
6.	Mr. MG.K.	48	105% (15.3)	39	50	78	Asthma, Bronchial.
7.	Mr. C.V.	53	99% (14.4)	41.2	48	90	Bronchial Asthma.
8.	Mr. J.C.	62	105% (15.3)	53.6	55	98	Chronci Pulmonary Fibrosis with cyanosis.
9.	Mr. J.C.	54	132% (19.2)	72.8	66	110	Severe Emphysema with cyanosis and Secondary Polycythemia.
10.	Mr. J.R.	78	104% (15gms.)	33.5	48	70	Emphysema. CO_2 combining power 67.3 vol. %.

Table 8 - Glutathione Content of Red Blood Corpuscles in Pulmonary Disease.

Mr. K.S.
Pulmonary Emphysema.

Date	Time	Comments	GSH in mgms.%	Hem.	Mgms.GSH/100cc. RBC's
22.4.53	8 A.M.	Had been on continuous O_2 for 6 days	38.8	59	66
24.4.53	8 A.M.	Taken off O_2 following this determination	39.2	55	71.3
24.4.53	9 A.M.	Taking O_2 only when absolutely required	40.4	58	69.7
24.4.53	11 A.M.	Taking O_2 only when absolutely required	39.6	56	70.7
24.4.53	2 P.M.	Taking O_2 only when absolutely required	38.6	55	70.2
25.4.53	8 A.M.	Taking O_2 only when absolutely required	43.6	55	79.3
26.4.53	8 A.M.	Taking O_2 only when absolutely required	47.2	59	80.0
27.4.53	8 A.M.	Taking O_2 only when absolutely required	47.2	59	80.0
28.4.53	8 A.M.	At 6 P.M. put back on continuous O_2	46.8	60	78.0
30.4.53	8 A.M.	On continuous O_2 since 6 P.M. Apr. 28 O_2 discontinued.	42.8	55	78.0
7.5.53	8 A.M.	Taking O_2 only when absolutely required	41.2	58	71.0
14.5.53			41.0	57	72.0

Table 9 - Glutathione Changes in Red Blood Corpuscles During the Administration and Deprivation of Oxygen Therapy in a case of Pulmonary Emphysema.

Mr. J.E. (No.1, Table 8)

Date	Comments	GSH in mgm.%	Hem.	Mgms.GSH/100cc. RBC
12.2.53	Markedly cyanotic and dyspnoea	49.2	48	102.5
20.2.53	Some Improvement	38.4	45	85.3
11.3.53	Markedly improved (no longer cyanotic)	22.8	39	58.4
17.3.53	Markedly improved (no longer cyanotic)	24.4	37	66.0

Table 10 - Changes in Glutathione Content of Red Blood Corpuscles following improvement in Pulmonary Function after Nitrogen Mustard Therapy for Bronchogenic Carcinoma.

Name	Age	Hg.	Diagnosis	GSH/mgms. %	Hem.	Mgms.GSH/100cc. RBC's
Miss N.MacD.	5	121%	Congenital Heart Disease no failure	35.0	52	67.3
Mrs. N.H.	60	69%	Prolonged Congestive Heart failure	29.6	31	98.1
Mrs. R.B.	48	74%	Mirtal Stanosis with Congestive heart failure	24.8	35	70.3
Mrs. I.P.	41	73%	Mitral Stanosis, auricular fibrillation. Right heart failure.	44.4	42	105.7
Mrs. C.A.	52	79%	Hypertensive heart disease with failure and uremia.	32.4	39	83.1

Table 11 - Glutathione Content of Erythrocytes in Patients with Heart Disease.

(see table 8) with values of glutathione/100 cc. red blood corpuscles ranging from 70 -110 mgms. with an average of 86.8 mgms. One case (see table 9) of pulmonary emphysema was studied during the administration and withdrawal of oxygen. It will be noted that following the withdrawal of oxygen, the glutathione content of the red blood corpuscles rose, associated also with an elevation in hematocrit. This did not occur within the first six hours but had occured within twenty-four hours. The rise might have been more rapid and more marked if he could have kept away from oxygen continuously. The return to continuous oxygen for thirty-six hours lowered both the glutathione content of blood (mgms.%) and the hematocrit, but proportionately, and there was therefore, no change in the glutathione content of the red blood corpuscles.

One case of improved pulmonary function, following nitrogen mustard therapy for bronchogenis carcinoma, was studied (see table 10). It is to be noted that there was a marked drop in the glutathione content of the erythrocytes associated also with a drop in the hematocrit. This drop in the glutathione content of erythrocytes was not due to the nitrogen mustard therapy, as will be shown later.

(3) Glutathione Content of Erythrocytes in Patients with Heart Disease.

Five cases of heart disease with failure were studied (see table 11). The values obtained ranged from 67.3 to 105.7 mgms. glutathione/100 cc. erythrocytes with an average of 85 mgms. for the five cases.

Name	GSH mgm.%	Hem.	Mgm. GSH/100 cc. RBC's	Comments
Baby McLeod	38.8	49	79	
Baby Sloan	51.2	57	90	Placenta small and fibrotic. Mother very toxic
Baby McLaughlin	45.2	55	82	
Baby Clement	43.2	47	92	Small baby cyanosed - small fibrotic placenta
Baby Campbell	43	52	83	
Baby New	29	46	63	
Baby Coombe	52.4	55	95	
Baby Paton	44.8	62	72	
Baby Danes	30.4	49	62	
Baby Hartley	36.8	57	65	

Table 12 - Glutathione Content of Erythrocytes of Cord Blood at Birth.

(4) Discussion.

Why should these conditions cause elevated glutathione levels in the erythrocytes ? In all these conditions the one common factor present, is the lowered oxygen carrying capacity of the blood, either because(1) there is a deficiency of hemoglobin as in the anemias; or (2) that the hemoglobin is unable to combine with oxygen as in the carbon monoxide poisoning; or (3) that there is poor oxygenation of the blood, as seen in certain forms of pulmonary disease, congestive heart failure and at high altitudes. Is the elevated glutathione level of erythrocytes to facilitate the oxygenation of hemoglobin, or the dissociation of oxygen from oxyhemoglobin, or is it to increase the efficiency of cellular metabolism under adverse conditions of oxygen pressure ? Handovsky (116) in 1930 stated that in conditions of oxygen deficiency there was an increased liberation of free glutathione, which would act as a hydrogen acceptor and oxidation would be replaced by dehydrogenation as a source of energy. Komissarov (117) states that in anemic hypoxemia, increased anaerobic oxidation occurs at the expense of glutathione and supplements the deficiency of oxidative processes. Gabbe (90) suggested that the glutathione content of erythrocytes was not only for the metabolic needs of the cells, but for assisting in the oxidation and reduction of hemoglobin.

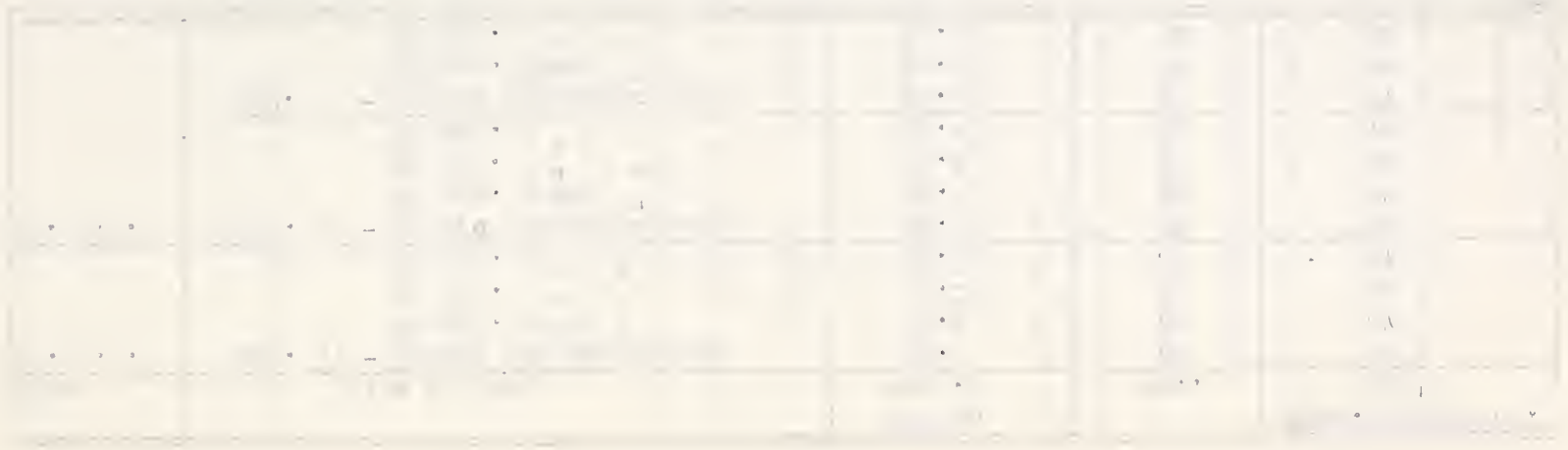
Hughes (118) has shown that crystallized oxyhemoglobin reacts with two moles of methyl mercury iodide/mole of hemoglobin at a pH 7.5. Ingbar and Kass (119) found that normal adult hemoglobin

has 2.0 SH groups/molecule. Riggs (120) states that a molecule of mammalian hemoblobin combines reversibly with four molecules of oxygen, one with each oxygen-combining centre or heme. These centres are all identical and are believed to be bound in the same manner to the protein, but when an oxygen molecule combines with one centre, it greatly increased the likelihood that a second oxygen will be attached to another. His investigation (120) indicates that the sulfhydryl (-SH) groups of hemoglobin are closely linked with the mechanism of heme-heme interaction in the oxygenation process. Hill and Wolvekamp (121) in 1936 found that glutathione appeared to make the oxygen equilibrium curve of a dilute hemoglobin solution slightly more highly inflected. McCarthy (122) and Hall (123) showed that foetal hemoglobin of goats and rabbits showed a greater affinity for oxygen than maternal hemoglobin. A study of the glutathione content of erythrocytes of cord blood at birth was made (see table 12). Of ten cases the glutathione content of 100 cc. of packed erythrocytes ranged from 62 to 92 mgms. with an average of 78.3 mgms. The two highest values obtained were from babies whose placenta was small and fibrotic. The foetus in utero exists in an atmosphere of low oxygen tension, and can be compared with the patient who has pulmonary disease or heart failure, in that it is difficult to oxygenate the hemoglobin. It was not possible at this time to compare the oxygen affinity of hemoglobin and the glutathione levels of erythrocytes in either the anemic patients, newborn, or in those suffering from pulmonary disease; however, it is postulated that

the glutathione levels of erythorcytes maybe a factor in increasing oxygen affinity of hemoglobin.

Name	Date		GSH in mgms.%	Hem.	Mgms.GSH/100 cc. RBC
Mr. D.M.	9.3.53	Nitrogen Mustard	24	39	62
#118420	10.3.53	10 mgms. I.V. daily	32.2	40	81
	11.3.53	on Mar. 9,10,11 & 12.	36.8	40	92
	12.3.53		36.4	38	96
Mr. N.H.	9.3.53	Nitrogen Mustard	26	43	60
#114928	11.3.53	10 mgms. L.V.daily on	37.6	49	77
		Mar.9, 10,11.			
Mr. A.D.	14.4.53	Nitrogen Mustard	30	46	67
#119564	15.4.53	10 mgms. I.V. daily	28.8	46	63
	16.4.53	from April 14 - 17 th.	30.0	46	67
	18.4.53		29.6	42	71
Mr. J.E.	11.3.53	Nitrogen Mustard	22.8	39	58
#117326	17.3.53	10 mgms. I.V. on	24.4	37	66
		Mar. 11,13.14,15.			
Mrs. M.H.	21.4.53	Triethylene melamine	24.8	34	73
#119283	23.4.53	(Lederle).	25.8	36	72
	21.5.53	Apr. 21 - 2.5 mgm.	27.2	39	70
		Apr. 29 - 5 mgms.			
		May 6 - 5 mgms.			
		May 12 - 7½ mgms.			
		May 19 - 7½ mgms.			

Table 13 - Glutathione (Content of Erythrocytes) During Nitrogen Mustard Therapy.



Name	Time & Date	GSH in mgms.%	Hem.	Mgms.GSH/100 cc. RBC's
Mr. J.E.	Mar. 11 - Before Administration	22.8	39	58
	20 min. After "	21.6	38	57
	40 min. " "	25.2	38	66
	60 min. " "	22.4	39	57
Mr. D.M.	Mar. 10 - Before Administration	31.6	38	83
	20 min. after "	34.0	35	87
	40 min. " "	31.2	37	84
	60 min. " "	25.6	35	73
	Mar. 11 - Before Administration	28.4	37	77
	60 min. After "	31.2	35	89
	80 min. " "	30.0	34	88

Table 14 - Gluthathione Content of Erythrocytes following 10 mgms. Nitrogen Mustard.

GLUTATHIONE CONTENT OF ERYTHROCYTES DURING NITROGEN MUSTARD THERAPY

The marked reduction of glutathione content of erythrocytes in the case of Mr.J.E.(see table 10) following nitrogen mustard therapy, indicated that nitrogen mustard should be ruled out as the etiology before one could definitely state that the lowering of glutathione content of erythrocytes in this case, was due to improved pulmonary function. The ability of B.A.L. to counteract mustard gas poisoning by virtue of its free -SH groups, and the use of nitrogen mustard in bronchogenic carcinoma, also suggested the possibility that the nitrogen mustard might lower the glutathione content of blood cells and tissues.

Four cases of nitrogen mustard therapy were followed (see table 13). In no case was there a lowering of the glutathione content of erythrocytes. Two of these cases (see table 14) were followed for periods up to eighty minutes after administration of the nitrogen mustard. Again there was no marked fall in glutathione content of erythrocytes. One case of triethylene melamine therapy (see table 12) showed no marked change in glutathione content even after one month of therapy.

Nitrogen Mustard therapy therefore, does not appear to lower the glutathione content of erythrocytes.

GLUTATHIONE AND DIABETES

Introduction

Jacobs (124) in 1937 found that alloxan when given intravenously to rabbits, caused a fatal hypoglycemia with convulsions. Bailey and Bailey (125) showed that actually, alloxan caused an initial hyperglycemia, followed by hypoglycemia with convulsions and death, but if glucose were given to prevent the convulsions, there developed symptoms seen in human diabetes. They (125) and others (126 - 130, 135) showed that alloxan caused marked degeneration and death of the beta cells of the islets of Langerans. Bailey and Leech (130) showed that cataracts developed in four to six weeks in rabbits made diabetics with alloxan. Brunschwig et al (129) - 131) demonstrated that alloxan, when given to a patient with an insulin producing islet cell carcinoma, gave temporary symptomatic relief from recurrent attacks of hyperinsulism. Others (132 -133) found that alloxan would control convulsions in children due to idiopathic hypoglycemia. Goldner (134),Gomori (136) and Bailey et al (137) showed, that when the vessels to the pancreas were clamped before, and for five minutes after the injection of alloxan, the beta cells in the clamped portion of the pancreas, did not undergo necrosis, whereas, the part not clamped off showed marked changes. It has been found that the pancreatic tissue of dogs made diabetic with alloxan, contains only one quarter the insulin content of the normal pancreas (134, 138) which is similar to dogs made diabetic by partial pancreatlectomy (139) by anterior pituitary extracts (140) and in human diabetes (141). The microscopic picture of other

forms of experimental diabetes e.g. pituitary diabetes (140,142, 143),that following partial pancreatectomy (144) and that following continuous glucose injections (145, 146) have been shown to be similar to those of alloxan diabetes; but with alloxan diabetes these changes occur more rapidly.

Leech and Bailey (147) reported that following the injection of alloxan the blood glutathione dropped rapidly to almost zero, and then returned to normal within 18 - 24 hours. They and others (148) reported the inability of glutathione and cysteine to protect animals against alloxan. Lazarow (149), however, in 1946 showed that the intravenous injection of large doses of glutathione or cysteine, one to two minutes prior to the injection of alloxan, caused complete protection, whereas, if given after the alloxan, only partial or no protection resulted, depending on the time interval. Palay and Lazarow (150) showed that where cysteine was used to protect rats from alloxan, there was a marked increased incidence of severe necrosis of the liver. This was explained on the possible chemical reaction between cysteine and alloxan giving dialuric acid and cystine; cystine having been shown to cause liver damage (151-153). Lazarow et al (154 -156) have shown that cysteine and alloxan give dialuric acid; and that glutathione and alloxan combine to form dialuric acid, and a substance which has an absorption spectrum giving a maximum at 305 mu. and which is thought to be an addition product of alloxan and glutathione. Bruckman and Wertheimer (157) showed dialuric acid was diabetogenic, but Archibald (158) has shown

that dialuric acid is rapidly converted to alloxan on standing in air, and their results could have been due to alloxan contamination.

Alloxan is an oxidative product of uric acid and it has been suggested that alloxan could possibly be formed during uric acid synthesis. Archibald (158) has shown that the blood of humans and dogs contained only minute amounts of alloxan (less than 0.02 mgms%). Griffiths (159) has shown that a transitory hyperglycemia could be produced in rabbits by uric acid injections; but only when they were kept on a diet deficient in methionine and cysteine, which caused a lowering of blood glutathione from 38 mgms.% to 18 - 23 mgms.%. He (160) suggests that the increased resistance of guinea pigs to alloxan as shown by Lazarow (149) and others (162) and in the common fowl Gallus domesticus (162) is due to their normally high blood levels of glutathione which are 58 mgms % and 60 mgms.% respectively in comparison with rabbits which are 35 - 40 mgms.%. Griffiths (160) reported guinea pigs were also susceptible to alloxan when kept on a diet deficient in methionine and cysteine, whereas, Grunert and Phillips (163) found that uric acid was non-diabetogenic in methionine and sodium deficient rats. Martinez et al (164,165, 166) observed that cysteine, thiouracil and 5-methylthiouracil given to rats for 12 to 30 days gave protection from alloxan and partial pancreatectomy and showed that protection was associated with an increased sulfhydryl concentration in liver and kidney. Methylthiouracil and propylthiouracil (167,168) thiouracil (169) and thiourea (170) have been reported to cause increased glutathione levels in blood.

Lazarow (171) showed that B.A.L. (Dimercaptopropanol) as well as cysteine and glutathione would protect animals against the development of alloxan diabetes; however, mole for mole, B.A.L. was at least twice as effective as the others. This was explained by the fact that B.A.L. contains two free -SH groups, whereas glutathione and cysteine contain only one. It is also evidence for the fact that it is the -SH group of glutathione which reacts with alloxan.

Du Vigneaud (172) has shown that insulin contains sulfur and that the sulfur was contained in a cystine molecule which is a disulfide linkage. Glutathione and cysteine (173) were found to inactivate insulin, probably by reduction of the disulfide linkage.

Glutathione-Dehydroascorbic Acid and Diabetes

Patterson (174) showed that injections of dehydroascorbic acid in rats lead to chronic hyperglycemia and glycosuria, Patterson and Lazarow (175) demonstrated protection by cysteine, glutathione and B.A.L. against dehydroascorbic acid intravenously if given before the dehydroascorbic acid, but not if given ten minutes afterwards. Banerjee (167, 177) had previously shown hypofunction of the islands of Langerhans in scorbutic guinea pigs as evidenced by diminished insulin content and degranulation of the beta cells. Banerjee and Ghosh (178) later found lowered glucose tolerance and decreased deposition of glycogen in the liver in scorbutic guinea pigs. It (179) has also been shown that there is a decreased glutathione content of the blood, adrenals, pancreas

and spleen of scorbutic guinea pigs with also the presence of dehydroascorbic acid in tissues in considerable amounts, which are normally not present. Banerjee et al (179) suggests the diminished insulin secretion in scorbutic guinea pigs, maybe due to the combined effects of low glutathione and high dehydroascorbic acid content of tissues in general and pancreas in particular.

Glutathione- Intermediary Fat Metabolism Products and Diabetes

It has long been recognized that obesity tends to increase the severity of diabetes, and with reduction in weight, diabetic patients can carry on with less insulin. Nath and Brahmachari (180) in 1944 showed that the injection of B hydroxibutyric acid, acetoacetic acid or pyruvic acid (intermediate fat metabolic products) caused elevated blood sugars and if continued for 150 days a permanent hyperglycemia ensued. They suggested that the pancreas was overstimulated by these products and became fatiqued by excessive work and later showed (181, 182) that these products were responsible for partial or complete inactivation of insulin, and felt that these ketone bodies were in the long run responsible for the developement of diabetes in many cases. Nath et al (183) have also reported that acetoacetate injections in rabbits caused a marked reduction in glucose tolerance, and also in glycogen storage of liver and muscle, both of which were difficult to correct with insulin. Sodium acetoacetate in-jections (184, 185) have been shown to cause increased blood lactate levels and decreased ascorbic acid levels. They (186,187) have found that

in rabbits acetoacetate injections cause the blood glutathione to fall, and repeated injections brought the blood glutathione to 0 in three to five days. A single injection has been shown to cause a drop in blood glutathione reaching a low in ninety minutes, and then rising to normal within three hours. Associated with the drop in glutathione there was a marked rise in blood sugar. They have (188) recently reported that these rabbits injected with sodium acetoacetate for five weeks are much more susceptible to alloxan than normal rabbits, and found that this increased susceptibility to alloxan was associated with a marked reduction in the glutathione content of the blood.

Lazarow (149) suggests that a low beta cell glutathione content would explain the selectivity of alloxan for the beta cells. He (189) also suggests that the synthesis of insulin in physiological amounts may produce a local depletion in beta cell glutathione, and thereby render these cells more susceptible to alloxan or to other sulfhydryl inactivators which may appear in the body. Glutathione is contained almost exclusively in the intracellular fluid, and its transportation and release must be fairly slow as we have mentioned the fact that alloxan causes the blood glutathione to drop almost to zero, and following this it requires 18 - 24 hours to return to normal. Beta cell degeneration is also observed in (1) partial pancreatectomy (190), (2) massive anterior pituitary hormone therapy, (140,142,191) (3) massive glucose injections (192,193). In all these conditions the beta cells are stimulated to an increased insulin

production. Lazarow (189) further postulates that this increased insulin synthesis also sensitizes the beta cells to degeneration, because of a consequent local depletion in beta cell glutathione. As he says "If this theory of beta cell degeneration proves correct, then the glutathione metabolism of the beta cells will not only affect the etiology of alloxan and other forms of experimental diabetes, but it may also have an important bearing on the development of human diabetes".

Previous Clinical Studies of Glutathione in Relation to Diabetes

The protection by glutathione against the production of alloxan diabetes and the fact that glutathione is a normal constituent of blood cells, and all tissues have stimulated many investigations of glutathione in relation to diabetes. Camanacci (194) and Varela et al (195) in 1930, long before the discovery of glutathione protection against alloxan diabetes, reported that the glutathione content of blood was decreased in diabetes mellitis, and Camanacci stated this decrease was independent of acidosis or blood sugar changes. Dogliotti and Meloni (196) in 1935 found normal blood levels of glutathione in diabetic patients.

Recently Stock and Currence (197) studied uric acid and glutathione content of the blood in diabetes. They found there was no deviation from the normal range in either blood reduced glutathione or in blood true uric acid. Caren and Carne (198) found that fasting blood glutathione values in diabetic patients treated with insulin

and those treated by diet alone were within the normal limits 25-41 mgms.%. They found no correlation between the blood sugar level and blood glutathione. Large doses of cystalline insulin varying between 100 and 580 units in physically normal schizophrenic patients caused no change in the blood glutathione. The blood glutathione was found to be within normal limits in a case of insulin resistance requiring 520 units insulin daily, and concluded that his insulin resistance was not due to inactivation of insulin by an abnormally elevated glutathione. They concluded therefore, that their results did not support the hypothsis of a diminished glutathione content of blood being responsible for the destruction or impaired function of the beta cells of the Islets of Langerhans in diabetes. Illing and Lawrence (199) found that the concentration of total glutathione in the diabetic without ketosis, was within the normal range, but observed that diabetics with ketosis had significantly lowered concentration of total glutathione, and that the reduced glutathione parallelled the values of total glutathione. Binkley et al (200) reported that, with their method, in an unselected group of diabetic patients, the concentration of glutathione was low, but this lowered value was compensated for by an increase in the concentration of y glutamylcysteine. Corrections for differences in hematocrit were not made in these studies.

	Name	Age	Acidosis	Serum Cholesterol mgms.%	Required Insulin dosage daily	Hemoglobin	Date	GSH in mgms.%	Hem.%	Mgm.GSH/100 cc. packed RBC	Comments & Associated Conditions.
1.	Mr.J.D.	60	No	218	None. Diet alone	110%	3.2.53	32.8	55	59.6	Slight cardiac enlargement.
2.	Mrs.S.M	57	No	313	NPH 15 units	110%	3.12.53	31.6	52	60.8	Bilateral Cataracts.
3.	Mr.J.S.	32	No		NPH 30,CZ 30	90%	2.1.53	36	46	78.3	Unstable diabetes.
4.	Mr.W.C.	45	No		PZ 30	102%	2.1.53	23.2	48	48.3	Previous Thyrotoxicisis. Now diabetes out of control. BMR -20 -25.
5.	Mr.A.J.	54	No		PZ 50 CZ 20 in a.m. CZ 15 at noon	104%	23.3.53	25.4	37	68.6	Furuncle and Cellulitis of neck.
6.	Mr.R.B.	71	No		Diet alone	95%	12.3.53	34.4	45	76.4	Serum Uric Acid 9.7 Mar. 9.53
							19.3.53	28.0	39	71.8	Serum Uric Acid 7.1 Mar. 11.53
											Serum Uric Acid 7.6 Mar. 19.53
7.	Mr.J.K.	86	No		Diet alone	85%	19.3.53	32.0	43	74.4	Bilateral Emphysema.
8.	Mrs.A.H.	64	No	247	Diet alone	97%	12.3.53	29.6	47	63	Bilateral Cataracts.
9.	Mr.R.B.	22	Yes Slight		PZ 30 units	104%	16.4.53	24.0	47	51.5	Untreated Diabetes.
10.	Mrs.R.E.	72	No		PZ 26 units	128%	13.1.53	36.8	64	57.5	Polycythemia Vera RBC 7,300,000.
11.	Mr.J.M.	27	No		30 unitsdaily	88%	10.12.53	27.8	42	66.2	Kimmelstiel Wilson Disease severe.
12.	Mr.A.P.	51	Yes		NPH 50 units	77%	19.2.53	35.4	45	78.7	Kimmelstiel - Wilson Disease.
							5.3.53	29.6	42	70.5	
13.	Mrs.D.H.	33	No		PZ 55 CZ 22	68%	15.1.53	23.6	32	73.8	Kimmelstiel-Wilson Disease. BUN 29.5 mgms.%. Urine 4 + protein.
14.	Mr.G.S.	25	No	760	PZ 60	104%	5.12.52	23.4	47	49.8	Kimmelstiel-Wilson Disease
							3.2.53	33.2	42	79.1	
15.	Mrs.L.S.	67	No		NPH 20 CZ 60	87%	19.11.52	32	43	74.3	Associated mild diabetic neuritis.
16.	Mrs.A.R.	48	No		Diet alone	92%	22.1.53	34.4	48	71.6	Mild diabetes.
17.	Mr.T.M.	53	No		PZ15 CZ 15 began 12.1. 53	59%	9.1.53	21.2	33	63.6	Untreated diabetes.
							12.1.53	21.6	35	62.1	Lowering of GSH in cells probably
							26.1.53	24.6	44	55.9	due to increased hematocrit.

18.	Mr.A.B.	36	No		PZ 20 units		15.1.53	24.0	45	53.3	Untreated Diabetes.
							22.1.53	28.2	47	60.0	
19.	Mrs.N.L.	53	No	160	Diet alone	93%	7.12.52	43.8	44	99.6	Untreated diabetes.Infected toe
20.	Mrs.E.O.	76	No		CZ 100 PZ50 b.i.d	98%	13.1.53	33.2	46	72.2	Resistant Diabetes.
21.	Mrs.E.P.	62	No		NPH 120 bid		14.5.53	33.2	45	74	Resistant Diabetes.
22.	Mrs.I.S.	40	No	181	Diet alone	66%	10.3.53	34.0	38	89.5	Mild diabetes. Associated anemia. Hypertension and vaginitis.
23.	Mr.S.P.	20	Yes		Discharged	99%	13.3.53	48.8	48	102	Untreated acidosis. Acetone. ++++
					on PZ 35		12.3.53	23.2	47	50.0	Acidosis - no
					CZ 25		26.3.53	22.4	46	48.7	Acidosis - no
24.	Mr.F.T.	13	No		NPH 40	86%	18.11.52	18.8	43	43.7	Very tall lanky boy for his age.
					CZ 80		20.11.52	21.0	44	47.7	Severe juvenile diabetes.
							26.11.52	21.2	45	47.1	
25.	Miss E.B.	14	No		Requiring 180 u.daily	92%	31.3.53	25.2	48	52.5	Previous pulmonary tuberculosis. Severe juvenile diabetes.
26.	Mr.A.B	64	No		NPH 30	84%	1.5.53	28.6	46	62.2	Diabetes developing during thyrotoxicosis.
							7.5.53	21.6	42	51.4	Thyroidectomy preformed 11.5.53
27.	Mrs.H.K.	63	No		PZ 20 units	84%	5.2.53	29.8	40	74.5	Previously anemic.
28.	Mr.S.J.	28	No	271	See Fig.6	89%	13.2.53	24.0	34	70.6	Anemia & Hypopthyroidism.
29.	Mrs. N.C.	75	No	200		59%	5.2.53	28.8	32	80	Anemia & Uremia
30.	Mr.J.P.	27	No			96%	13.1.53	34.8	48	72.5	Cushing's Syndrome.
31.	Mr.N.S.	58	No		PZ 16 units	89%	24.3.53	21.6	46	47	Hemochromatosis Adrenocortical Insufficiency.

Table 15 - Glutathione Content of Erythrocytes in Patients with Diabetes Mellitis.

Age	y = a + bx	Mgms.GSH/100 cc. RBC's
5	53.9 + 0.2494 x 5	55.15
20	53.9 + 0.2494 x 20	59.89
40	53.9 + 0.2494 x 40	63.68
60	53.9 + 0.2494 x 60	68.86
80	53.9 + 0.2494 x 80	73.65

Table 16 - Statistical values of the Regression Line of mgms. Glutathione /100 cc. packed cells against Age in Cases of diabetes mellitis where y is glutathione content and x is age in years (a = 53.9).

RESULT OF THIS STUDY OF GLUTATHIONE IN RELATION TO DIABETES

In this study thirty one cases of diabetes mellitis were followed (see table 15). Milligrams glutathione/100 cc. packed erythrocytes varied from 43.7 to 102 mgms. A statisical study of twenty eight of these cases (3 cases No.22,29 and 31, see table 15, were omitted because of associated anemia) showed on average age of 47.2 years with an average glutathione value of 65.7 mgms./100 cc. packed erythrocytes. These cases, as the normal individual did, showed an increase of glutathione content with age (see table 16). The value of "t" is 2.32 with 26 degrees of freedom. The probability of deviation from 0 as great as that found was 0.03, which is significant at the 5% level. A comparison of table 16 and table 4 appears to show that the slope of the regression line is greater for the diabetic than the normal, but the difference is not statistically significant, "t" for the difference in the two lines being 1.263. Part of the difference in the slope of the two lines could be due to the fact that two cases of mild anemia and one case of pulmonary emphysema as associated conditions were included in this series.

In this study it should be noted there were two cases of resistant diabetes. The glutathione content of erythrocytes fell within normal limits in both cases. Two cases of severe juvenile diabetes were studied. In one case the glutathione content of the erythrocytes was below normal limits on three different occasions, while in the other case it was at the lower limits of normal.

There were four cases of diabetes mellitis with associated Kimmelstiel/Wilson's Disease, and two cases of bilateral cataracts associated with diabetes mellitis. Glutathione content of erythrocytes in these cases did not show any deviation from normal.

It appears therefore that a clinical study of diabetes mellitis in relation to glutathione content of erythrocytes does not suggest or indicate any correlation between the two. There is no suggestive evidence here to associate a low glutathione content of erythrocytes in patients with diabetes mellitis as part of the etiology of diabetes mellitis unless in juvenile diabetes. The two cases of juvenile diabetes studied here showed low glutathione levels in the erythrocytes, but more cases would have to be studied before a definite conclusion could be drawn.

The initial high value of glutathione content of erythrocytes in the case of Mr.S.P.(table 15) was probably due to ketone bodies present in blood and the method is not considered accurate for a person in diabetic acidoses (see table 3). Estimation of glutathione taken two days later, when acetone had disappeared from the urine, showed values much lower than when he was first admitted.

GLUTATHIONE LEVELS IN RELATION TO PITUITARY DISEASES

The anterior pituitary extracts have been shown to cause diabetes, (140,201 -210) which can be temporary or permanent, depending on dose, length of time of giving, and personal susceptibility, and changes in the pancreas (140,142,143,191.211,212). Atkinson (213) showed that glyosuria was found in 33% of his cases of acromegaly. Ennor (214) and Goss and Gregory (215) reported that anterior pituitary extracts and hypophyseal growth hormone respectively, caused decreased glutathione content of the liver. Conn et al (202, 206) found decreased levels of glutathione in blood associated with the developement of a resistant form of diabetes, with high uric acid levels in blood and increased urinary execretion of uric acid on ACTH therapy. Hess, Kyle Doolan (205) found that ACTH caused decreased reduced glutathione levels whereas oxidized glutathione did not change. The greatest decrease of glutathione was associated with the greatest elevation of blood sugar. Kass et al (216) in a study of one patient on ACTH found diminished blood glutathione levels. Levine and Adams (207) studied ACTH therapy in Periarteritis Nodosa and found that ACTH lowered the blood glutathione levels. Ingbar et al (217) found no change in blood glutathione levels following ACTH therapy in rats. Grunert and Phillips (218) also found no changes in glutathione content of blood of rats given single and repeated injections of ACTH. Joiner (208) studied blood glutathione levels in eleven cases of ACTH therapy in humans, and found no change, even though carbohydrate intolerance developed.

Mr. M.L.

Day	FAsting Blood Sugar mgms.%	½ hr. Blood sugar mgms.%	1 hr. Blood sugar mgms.%	1½ hrs. Blood sugar mgms.%	2 hrs. Blood sugar mgms.%	2½ hrs. Blood sugar mgms.%	Serum Uric acid mgms.%
0	76	80	95	94	34	53	5.1
5	67	147	136	142	102	62	4.7
12	68	101	61	98	36	54	

Table 17 - Changes in Glucose Tolerance Test and Serum Uric Acid Levels as a Result of ACTH Therapy (25 mgms. I.V. daily) for 5 days.

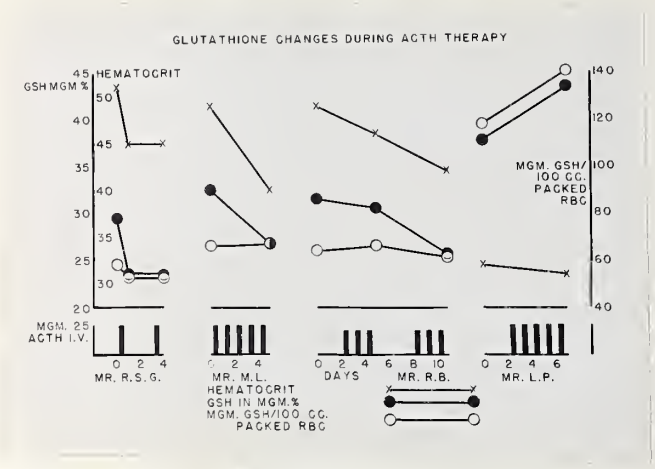

GLUTATHIONE CHANGES DURING ACTH THERAPY
HEMATOCRIT
GSH MGM %
MGM. GSH/ 100 CC. PACKED RBC
MGM. 25 ACTH I.V.
MR. R.S.G.
MR. M.L.
MR. R.B.
MR. L.P.
DAYS
HEMATOCRIT
GSH IN MGM.%
MGM. GSH/100 CC. PACKED RBC

Figure 4

Holton and Lundback (201) have reported no change in blood glutathione levels in two men receiveing ACTH even though they developed abnormal glucose tolerance curves, and lowered renal threshold to glucose. Hennaman and Altschule (219) in comparison with the others, found that ACTH given to psychotic patients caused elevation of blood reduced glutathione, two to four hours after, expressed in terms of its concentration in erythrocytes.

Conn et al (220), gave reduced glutathione intravenously to patients receiving ACTH therapy. and found that reduced glutathione caused a sharp elevation of renal threshold for glucose, and a fall in blood sugar levels which lasted for one to two hours.

In this study, four cases of ACTH therapy were followed (fig.4) The first two cases (Mr. R.S.G. and Mr.M.L.) were given ACTH for ecaema. The third case (Mr. R.B.) had pulmonary fibrosis. The fourth case (Mr. L.P.) had severe rheumatoid arthritis and had been taking cortisone 100 mgms. daily for one year, resulting in marked hypofunction of the adrenal cortex, and ACTH was given to stimulate the adrenal cortex. Mr.M.L. was the only case in which the glucose tolerance and serum uric acid levels were followed (see table 17). It will be seen he did develop an abnormal glucose tolerance although serum uric acid values did not change. It is to be noted that in all four cases there was a drop in the blood hematocrit, most marked in the first three. Associated with this drop in hematocrit there was a corresponding drop in glutathione content of blood (mgms.%), but when corrected for the change

in hematocrit the glutathione content of the erythrocytes had not changed appreciably. In the case of Mr.L.P. there even was a marked increase in the glutathione content of erythrocytes during ACTH therapy. It should be noted that except for Henneman and Altschile (219) the others have not considered changes in hematocrit in reporting their results, and it is quite evident from the results reported here that the drop in glutathione content of blood, as reported by others, is due mostly to a state of hydremia, which developes during ACTH therapy, with a resultant drop in hematocrit. ACTH therapy does appear to lower the glutathione content of erythrocytes.

One case of pituitary insufficiency due to chromophobe adenoma of the pituitary was studied and the glutathione content of the erythrocytes was found to be within normal limits (see appendix).

GLUTATHIONE LEVELS IN RELATION TO CONDITIONS ASSOCIATED WITH THE ADRENAL CORTEX

The adrenal cortical hormones have beeen shown to eause diabetes (205,221,226). Lukens et al (227) have reported a case of adrenal cortical tumor with diabetes, and Sprague et al (228) reported a case of a woman whose only manifestation of a malignant adrenal cortical tumor was diabetes, and whose diabetes disappeared on removal of the tumor. Russi et al (229) reported that diabetes occurs five times as frequently in individuals having adrenal cortical adenomas as it does in the general autopsy group. Binet and Poutonnet (230,231) reported low values of reduced and total glutathione in Addison's Disease, and found that adrenal cortical extracts or DOCA brought about a return to normal values. In comparison Hess et al (205) reported that cortisone caused a reduction in reduced glutathione, whereas oxidized glutathione did not change. Lazarow and Berman (223) showed that cortisone injected into rats caused a significant drop in blood glutathione levels. Lazarow (222-224) showed that where, either rats fed a high carbohydrate diet with the developement of glycosuria, or where glycosuria developed in rats during cortisone therapy, the injection of glutathione caused an intensification of the glucosuria associated with elevated blood glucose levels. As the rats became adapted to a high carbohydrate diet, however glutathione caused only an insignificant glycosuria. Glutathione caused death to rats with severe cortisone diabetes. In comparison glutathione to normal animals in much larger doses caused no change in blood sugar or glycosuria and when given to

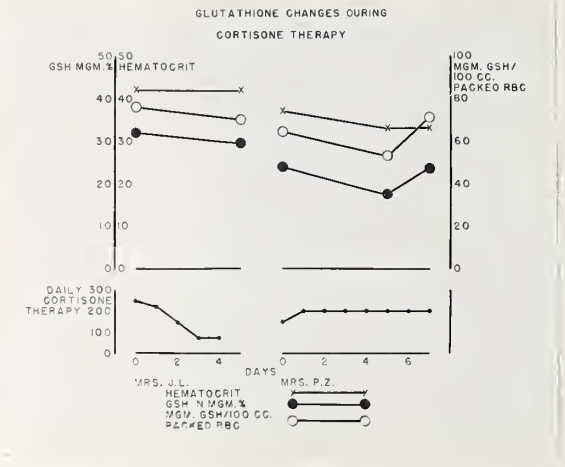

GLUTATHIONE CHANGES DURING
CORTISONE THERAPY
GSH MGM.%
HEMATOGRIT
MGM. GSH/
100 CC.
PACKED RBC
DAILY 300
CORTISONE
THERAPY 200
100
0
0 2 4
DAYS
0 2 4 6
MRS. J.L.
MRS. P.Z.
HEMATOCRIT
GSH IN MGM.%
MGM. GSH/100 CC.
PACKED RBC

Figure 5

severe alloxan diabetic rats it did not increase the glycosuria. He emphasizes the fact that although glutathione may protect the beta cells of the pancreas against physiologically occurring substances, it may also have diabetogenic effects due to its action on steriods and enzyme systems. The results of Lazarow et al with glutathione in rats made diabetic with cortisone should be compared with the results of Conn et al (202,206,220), using glutathione in diabetes caused by ACTH.

Two cases of cortisone therapy were followed (see figure 5). Mrs.J.L.had multiple neurofibromatosis and Mrs.P.L. had rheumatoid arthritis. In the case of Mrs.J.L. the glutathione content of 100 cc. of packed erythrocytes fell from 76.2 mgms. to 70 mgms. which is felt to be insignificant. In the case of Mrs.PZ. there was a slight drop in glutathione content of the erythrocytes at the 5th day, but on the 7th day it had become elevated above the initial level. It should be noted again that previous investigators of cortisone therapy have failed to correct for any change in hematocrit which might occur. The results shown here do not show any marked fall in glutathione content of the erythrocytes as a result of cortisone therapy.

One case of Cushing's Syndrome was studied (see table 18). The glutathione content of red blood cells was found to be within normal limits.

Four cases of Adrenogenital Syndrome were studied (see table 18). Two of these showed values within normal limits, and two would

Disease	Name	Age	Hb.	24 hr. 17 Ketosteroid excretion (mgm.)	24 hr. corticoid excretion (mgm.)	Dehydroisoandriosterone	Glucose Tolerance	GSH in mgm.%	Hematocrit	Mgm.GSH/100 cc. packed RBC	Comments and other findings
Cushing's Syndrome	Mr.J.P.	27	90	39.7	2.12	Not Done	Abnormal	34.8	48	73	Plethoric individual with obesity of face, neck and trunk. Bluish striae of skin. B.P, 160/120. Marked osteoporosis.
Adrenogenital Syndrome	Mrs.A.E.	26	86	25.13	1.83	Neg.	Not Done	37.8	47	80	Plethoric moon face with obesity of the trunk. Purple striae over flanks. B.P. 140/110. Hirsutism.
Adrenogenital Syndrome	Mrs. J.D.	23	93	28.99	0.89	Neg.	Normal	33.8	47	72	Round hirsutic face and obese torso. B.P. 120/70.
Adrenogenital Syndrome	Master R.C.	10		17.5			NotDone	35.4	51	69	Deep voice, axillary and pubic hair. Operation showed small uterus, tubes and ovaries. Left adrenal very large.
Adrenogenital Syndrome	Baby L.W.	20 mons.						37.8 31.4	41 41	92 77	4 inches taller and 9 pounds heavier than 2 year old child.

Table 18 - Glutathione Content of Erythrocytes in Patients showing Hyperfunction of the Adrenal Cortex.

be considered high, however a later determination on L.W., age twenty months, showed that the glutathione content of erythrocytes had decreased somewhat.

One case of adrenal cortical insufficiency (see appendix) showed values within normal limits.

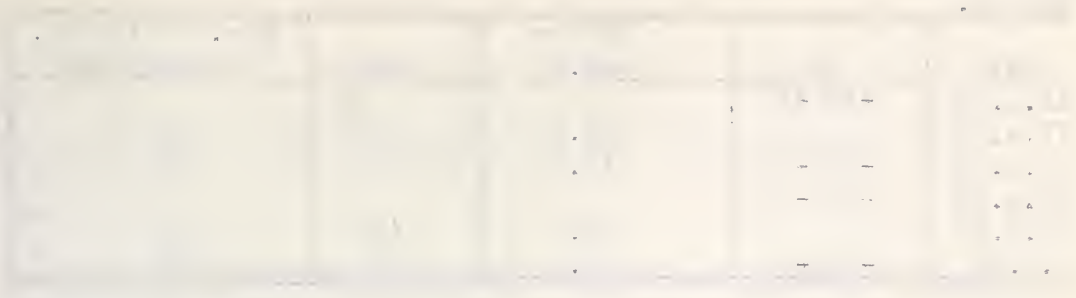
Even though the number of cases studied here are small it does not appear that the hormones of the adrenal cortex have any marked effect on the glutathione content of erythrocytes, as has been reported previously.

GLUTATHIONE CONTENT OF ERYTHROCYTES IN RELATION TO DISEASES OF THE THYROID GLAND

Maloberti (232, 233) in 1935, found lowered glutathione blood levels in humans with hypothyroidism. Braier(234) in 1936 showed, that in rabbits, rats and guinea pigs, the injection of thyroxine caused a decrease of 20% in total glutathione in the blood, with a 40% increase in the liver and in the heart and 30% increase in the lungs and spleen. Zunz and Vesselovsky (235) in 1938 found no change in blood glutathione levels in dogs after intravenous thyroxine injections, whereas Livierados (236) in the same year found that in dogs given 2 mgms. thyroxine/kh body weight there was a transient decrease in glutathione. He also found that thyroidectomy in dogs caused a 50 - 100% increase in blood glutathione levels. Coccialanza (237) in 1939 also reported that in dogs the blood glutathione rose following thyroidectomy. In 1943 Mutel (238) showed that the total blood glutathione decreased in proportion to the increase in the basal metabolic rate. Capra (239) in 1947 studied patients whose metabolic rate was increased with thyroxine and found that the glutathione content of blood was decreased. He (167. 168. 239) also studied the effects of methylthiouracil and propylthiouracil in normal paitient and those with thyrotoxicosis and found that the blood glutathione varied inversely to the basal metabolic rate; Prina (169) reported that thiouracil given to rabbits for one month caused a moderate increase in both reduced and total glutathione content of the blood. Domingo (170) giving thiourea to patients with tuber-

Name	Age	BMR	Serum Cholesterol mgm.%	Hemoglobin	Comments and Associated Conditions	GSH mgm.%	Hem.	Mgm.GSH/100cc RBC'C
Mr. N.M.	18	+29 +32	75	88%	WBC 6,950 with only 19% polymorphonucears. See table 19.	14.8	42	35
Mr.K.S.	37	+66 +58	132	102%	Propylthiouracil 200 mgms. tid began 4 days previously	32.0	46	70
Mr. J.H.	51	+41 +43	120	96%	Radio active Iodine theraphy. Second glutathione value following therapy.	22.4 26.0	40 41	56 63
Mr. E.N.	48	+43 +40	305	89%	Took Propylthiouracil 300 mgms. daily for 10 mons. Stopped 2 mons. ago because of exophthalmos. Taking iodine alone Since then had had recurrence of Thyrotoxicosis.	28.6	47	61
Miss E.C	33	+53 +53		84%	See table 20	19.6	38	51
Mrs. C.W.	44	+10 +14		92%	Clinically mild thyrotoxicosis.	27.6	41	67

Table 19 - Glutathione Content of Erythrocytes in Patients with Thyrotoxicosis.



Mr. N.M
Age 18.

Date	BMR	GSH in mgm.%	Hem.	Mgm.GSH/100 cc. packed RBC
14.4.53	+47 +47			
15.4.53		14.8	42	35
16.4.53	+29 +32	17.2	43	40
22.4.53	+36 +33			
23.4.53		22.0	45	49
7.5.53	+24 +24	15.4	42	37

Table 20 - Glutathione Content of Erythrocytes During Tapazole Therapy for Acute Thyrotoxicosis.

Miss E.C.
Age 33

Date	BMR	GSH in mgms.%	Hem.	Mgm.GSH/100 cc. packed RBC'C
21.1.53	-53 -53			
29.1.53		19.6	38	51
30.1.53	+33 +33	16.6	42	40
11.2.53	+29 +24			
24.2.53	+15 +12	20.0	42	48
26.2.53		22.0	42	52
10.3.53	-1 -1			
17.3.53		23.4	44	53
18.3.53		Thyroidectomy Performed.		
26.3.53		20.8	45	46
23.4.53		22.4	42	53

Table 21 - Changes in Glutathione Content of Erythrocytes During Tapazole Therapy for Acute Thyrotoxicosis.

Name	Age	BMR	Cholesterol mgm.%	Hg.	GSH mgm. %	Mgm.GSH/loocc. Hem.	RBC
Mrs.E.R	47	-26 -28	315	97%	32.4	46	70
Mr.E.D.	39	-40 -42	447	66%	28.8	31	93
Mr.S.J.	28	-42 -40	271	80%	24.0	34	71

Table 22 - Glutathione Content of Erythrocytes in Patients with Hypothyroidism.

culosis and increased basal metabolic rates found that the blood glutathione content of blood increased as the basal metabolic rate decreased.

Six cases of thyrotoxicosis were studied (see table 19). The average age was 38.5 years with an average glutathione content of 57.6 mgms./100 cc. packed erythrocytes. Two of these patients were followed during therapy with tapazole. (see tables 20 and 21) The changes in glutathione content of erythrocytes did not appear to change significantly in either of these two cases, even though one case, Miss E.C. (table 21) reached a stage of euthyroidism and was followed even after thyroidectomy had been performed. It appears from the six cases that the erythrocytes of people with thyrotoxicosis contain less glutathione than normal individuals. It is interesting to note, however, that there did not appear to be any marked increase in glutathione content whereas there was clinical improvement. One case, Mr. J.H. (see table 19) who had radioactive iodine therapy in Winnipeg, and who had noticed marked personal improvement for about four days, showed an increase in glutathione content of erythrocytes from 56 to 63 mgms/100 cc. packed erythrocytes, which may or may not be significant.

Three cases of hypothyroidism were studied (see table 22). The average age was twenty-eight years with an average glutathione content of 78 mgms./100 cc. packed erythrocytes. This high average value appears to be due to the associated anemia present, especially in the case of Mr.S.J. Two of the three cases (Mr.E.D. and Mr.S.J.

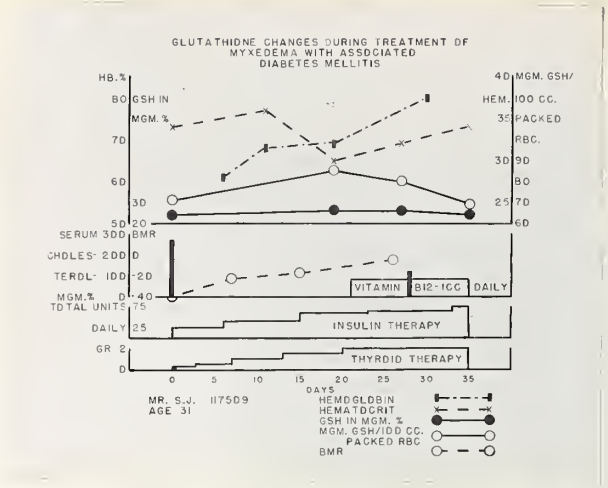


Figure 6

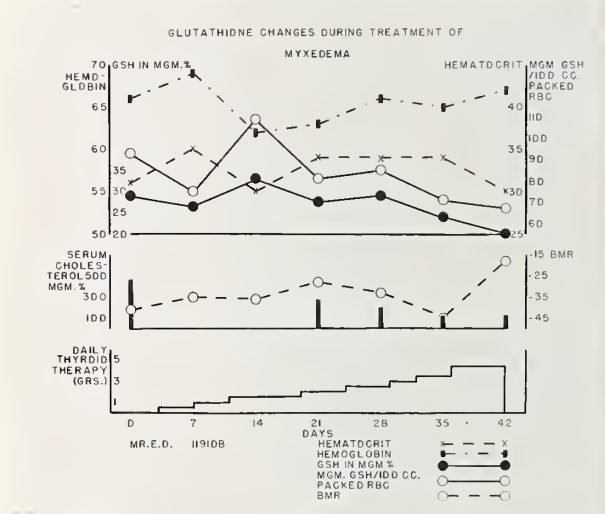


Figure 7

were definitely myxoedematous, and Mr S.J. had suffered from diabetes mellitis. Figure 6 shows glutathione changes occurring during treatment of Mr.S.J. with dessicated thyroid. It should be noted, that, as the basal metabolic rate returned towards normal, the requirement for insulin increased, and there was a drop in serum cholesterol. Glutathione content of erythrocytes did not appear to change appreciably, and any change was inversely proportional to the change in hematocrit. Figure 7 shows the changes in glutathione content of erythrocytes in the case of Mr.E.D. It will be noted again that the glutathione content of erythrocytes appears to vary inversely proportionally to the hemoglobin and hematocrit, but with the increasing dosage of dessicated thyroid there appeared to be some lowering of the glutathione content, even though there was a decrease in hematocrit. Mention should be made however, that even though the hematocrit decreased slightly, at the end, the h maglobin showed an increase. Whether this is a true case of hypothyroidism or whether there is some hypofunction of the pituitary gland is hard to assess. Cortin and Ketosteroid assays initially, gave values of 0.90 and 7.10 mgms./24 hours respectively. Rechecks taken six weeks later gave values of 0.46 and 6.34 mgm./24 hours. It is felt however, that if this case was primarily hypopituitary, he would have shown evidene of this on thyroid therapy. In these two cases dessicated thyroid did not appear to alter the glutathione content of erythrocytes significantly.

Name	Age	Etiology	Date	Total Serum Bilirulium mgms.%	Direct Serum Bilirulium mgms.%	Ceph. Flocculation in 48 hr.	Prothrombin time.	Thymol Turbidity	Comments	Date	GSH in mgms.%	Hem.	Mgms.GSH/100 cc. RBC's
Mr.N.S. Gen.Hosp.	64	Post-transfusion	31.12.52 2.1.53	18.4 28.4					Semicomatose Died 16.1.53	6.1.53	23.2	43	54
Mr.H.L.J. #50841	46	Post -transfusion	19.10.52 22.10.52 25.10.52 30.10.52 7.11.52 14.11.52 1.12.52 15.12.52	10.5 18.8 17.2 9.34 4.6 3.13 1.65 1.13	6.6 11.8 11.6 4.77 2.46 1.76 0.81 0.43	++++ ++++ ++		 4.5 u 2.8 u	Associated Diabetes Mellitis	3.11.52 5.11.52 14.11.52 1.12.52 15.12.52	24.6 26.2 26.0 28.4 25.6	43 38 41 44 40	57 69 63 65 64
MissG.P. #102820	17	Post -transfusion	3.10.52 5.12.52	11.5 0.86	8.3 0.25	+++		 1.6 u	Recovered when GSH taken	5.12.52	24.2	42	58
Mr.N.S #116611	26	Infectious	29.12.52	10.3	6.47					30.12.52 2.1.53	38 40.4	56 54	68 75
Miss S.B. #106997	21	Infectious	11.12.52 18.12.52			++ ++		15 u		5.12.52 18.12.52 23.4.53	24.4 25.5 26.0	45 44 41	54 58 63
Mrs.G.S. #2320	33	Infectious	28.10.52 10.11.52 1.12.52 15.12.52	7.7 2.37 0.77 0.31	4.6 0.82 0.34 0.11	+++	24%	 8.2 u 4.8 u		6.11.52 17.11.52 1.12.52 15.12.52	30.0 27.2 23.9 26.0	43 39 40 39	70 70 60 67
Mr.P.B. #114983	24	Infectious	31.10.52 4.11.52	6.13 3.60	3.35 1.97					4.11.52	24.6	46	54

Table 23 - Glutathione Content of Erythrocytes. Acute Virus Hepatitis.

GLUTATHIONE CONTENT OF ERYTHROCYTES IN RELATION TO LIVER DISEASE

The incorporation of glycine, cysteine and glumatic acid into glutathione ((9 -15) by liver slices has stimulated investigations of glutathione in relation to the liver. Protein free diets have been shown to cause marked depletion of liver glutathione (17,241, 242), although no lowering of blood glutathione was found. Liver necrosis has been reported (17,242) on these diets, and it was felt (242) that this is probably due to some toxic agent to which the organism is more susceptible to with a reduction in liver glutathione. Brunschwig et al (243) reported that glutathione injected intraperitoneally in animals gave appreciable portection against carbon tetrachloride poisoning. Cinelli (240) reported lowered glutathione content of blood in mgms.% in obstructive liver disease. Binkley et al (80) state that the glutathione content of blood has been reported to fall in hepatectomized animals and in animals with acute hepatic disease;

Glutathione content of erythrocytes was studied in relation to acute hepatitis and other forms of liver disease. There were seven cases of acute hepatitis, (see table 23) three due to transfustions of blood or plasma, and four due to infectious hepatitis. In only one case, Mr.H.S. could one say that the glutathione content of erythrocytes w was decreased. He was in a state of cholemia when the determination was made and died four days later. In the case of Miss S.B., a nurse in hospital, and who was recognised very early, there was an indication

Name	Age	Etiology	Date	HB.	Serum Protein gms.%	Albumen gms.%	Globubin gms.%	Prothrombin Time %'age of normal	Total serum Bilirulium mgms.%	Direct serum Bilirulium mgms.%	Cephalin Flocculation 48 hrs.	Comments	Date	GSH mgms.%	Hem.	Mgms.GSH/100 cc. RBC's
Mrs.G.B. #103695	22	Lympho-sarcoma	25.3.53	8.2gms	6.58	3.81	2.77	8%	22.9	13		Began x-ray therapy 27.3.53	26.3.53 2.4.53 9.3.53	16.8 14 13.2	38 32 31	44 44 43
Mrs.L.G. #116128	62	Obstructive Jaundice. Tumour of Ampulla of Vater										Cholecysto-jejunostomy 16.12.52	23.12.52 2.1.53	21.6 20.2	45 41	48 49
Mrs. U. 114761	71	Liver metatasis by adenocarcinoma.Primary Unknown.	5.11.53		8.33	3.28	5.05		1.79	125			5.11.52	24	42	57
Mr.T.McI 10103	63	Marked Cirrhosis of the liver			8.50	3.65	4.85	52%			- ++		15.12.52	34.2	53	65
Mr.M.E. 106274	21	Severe Obstructive Jaundice with bilary cirrhosis since 1950						55%	23	14.8	++	Draining biliary fistula attacks of chills & fever. Enlarged liver.	6.11.52	21.6	41	53
Mr.J.R. 115616	12	Cirrhosis of the liver. Etiology unkown.	17.11.52 1.12.52		6.30 6.30	1.91 1.59	4.39 4.71	29%	2.61 1.03	0.55 0.45	++++ ++++	Marked Ascites	24.11.52 5.12.52	21.8 23.4	48 42	43 56

Table 24 - Glutathione Content of Erythrocytes in Liver Diseases other than of Viral Origin.

of an increase in glutathione content of erythrocytes four months later, when she had returned to work. In the other cases no definite change in glutathione content cou ld be shown. There were six cases of liver disease other than acute hepatis followed (see table 24). Four of the six cases showed low glutathione content of erythrocytes for their age, although one case , Mr.J.R., did later show a rise in glutathione content when there was also evidence of clinical improvement.

LEUKEMIA

Glutathione has been studied in relation to leukemias. Platt (100) reported high values of glutathione in the blood of patients with myelogenous leukemia. These values dropped to normal in whole blood following X-ray therapy. In chronic lymphatic leukemia he reported only a doubtful increase, whereas in acute lymphatic leukemia he found high values. Malenkova (244) also found higher glutathione values in patients with leukemia, and thought most of the increase was in the leucocytes. Bickel (94) found a reactionary leucocytosis to 30,000 gave no appreciable change in glutathione values. Binet et al (245) found that reduced blood glutathione was consistently elevated in acute leukemia. They reported lowered values during cortisone therapy, but aminopterin, nitrogen mustard and blood transfusions did not cause this effect. Contopoulis and Anderson (246) have successfully been able to separate leucocytes from erythrocytes. In leukemias they found markedly elevated glutathione content of leucocytes, especially in acute lymphatic leukemia. X-ray therapy they found caused a decreased glutathione content of leucocytes in myelogenous leukemias. Two cases of myelogenous leukemia were studied (see table 25). The first case Mr,A.T. showed a picture of acute myelogenous leukemia and died shortly afterwards, so could not be followed. The second case, Mrs. W.Mc.D, had received a great deal of X-ray therapy, and the bone marrow and blood smear had returned to normal. It is interesting to note that even though she had a severe anemia her glutathione

Name	Age	WBC	Hg.	Hem.	GSH In Mgm.%	Mgm. GSH/100cc. RBC
Mr. A.F.	36	37,000	32%	17	31.2	184 mgm.
Mrs.N.McD	61	3,200	10%	10	5.2	52 mgm.
				14	11.9	85 mgm.

Table 25 - Glutathione Content of Blood in Myelogenous Leukaemia.

Date	Hb%	WBC	GSH in Mgm.%	Hem.	Mgms.GSH/100 cc. Packed RBC
24.2.53	68	76,000	37.6	31	121
26.2.53	68	77,000	36.2	36	101
3.3.53		30,000	35.4	36	98
5.3.53	68	27,000	34.8	36	97
16.4.53	83	4,100	26.8	44	61

Table 26 - Glutathione Content of Blood and Erythrocytes during x-ray therapy in a Case of Chronic Lymphatic Leukaemia.

content was below the normal limits for her age, and the second determination , which was taken two days later, during which time she had received two blood transfustions, was much higher, This could either be due to an error in the method, as at this level (5.2 mgms.%) the accuracy of the method is to be questioned, or this could be due to receiving normal erythrocytes by transfusion which had not received X-ray therapy, which could take up or manufacture their own glutathione. One most realize that we are measuring erythrocytes and leucocytes together, and not differentiating between the two; but the case of acute myelogenous leukemia did show very high values.

One case of chronic lymphatic leukemia (see table 26) showed high levels of glutathione. It is interesting to note that even though there was a drop in white blood count with X-ray therapy from 77,000 to 27,000 there was not a significant change in glutathione content, but later, with an increase in hemoglobin and hematocrit, there was a marked decrease in glutathione content. It must be realized that we are again not separating the leucocytes from the erythrocytes, but when there was no marked fall in glutathione content initially with a marked fall in leucocyte count, the marked fall in glutathione content can probably be ascribed to the elevation in hematocrit and hemoglobin.

Any worthwhile study of glutathione in relation to leukemia would require a seperation of the leucocytes from the erythrocytes, as Contopoulis and Anderson (246) have accomplished, and then a measure of glutathione content of each separately.

Name	Ethiology & Comments	WBC	PMN%	LYmph %	Hg.	Date	GSH mgms. %	Hem.	Mgm.GSH/100cc. RBC
Mr. J.S.	Infectious Mononucleosis. Temp 101 degrees, age 20	21,050	37%	61%	103%	13.2.53	30.4	49	62
						17.2.53	34	51	66
						17.3.53	33	50	64
Mr. A.R.	Measles. Temp. 104 degrees. Age 22.	5,400	84%	16%	104%	8.4.53	28.2	42	67
Miss L.J.	Subdiaphragmatic Abscess. Temp. 102 degrees. Sed. Rate 41, age 21.	7,050	58%	42%	75%	3.2.53	26.8	35	77

Table 27 - Glutathione Content of Erythrocytes in Patients with Acute Pyrexia.

Name	Age	Associated Conditions	ESR	Hg.	GSHmgms. %	Hem.	Mgm.GSH/100 cc. RBC's packed
Mrs. PZ.	75	Rheumatoid Arthritis.	35	73%	24.2	37	65
Mr. P.G.	50	Chronic Osteomyelitis.	44	96%	36	43	84
Mr. J.W.	22	Probably subacute Bacterial Endocarditis.	38	84	21.2	36	59
Miss M.D.	7	Behaviour Problem	26	80%	34.4	37	93
Mr. C.McC	30	Recurrent Diarrhoea. Arthritis. Erythema Multiforme.	37	70%	39.6	36	110
				89%	35.6	42	85
Miss L.W.	22	Acute Rheumatic Fever	40	84%	26.0	35	74
Mrs. E.R.	53	Erythema. Nodosum	53	92%	27.2	37	73
Mr.D.D.	60	Rheumatoid Arthritis	45	92%	38.4	46	84
Miss L.J.	21	Subdiaphragmatic Abscess	41	75%	26.8	35	77
Mr. L.P.	39	Rheumatoid Arthritis.	44	40%	40.0	31	129

Table 28 - Glutathione Content of Erythrocytes in Diseases Associated with Elevated Erythrocyte Sedimentation Rates.

PYREXIA

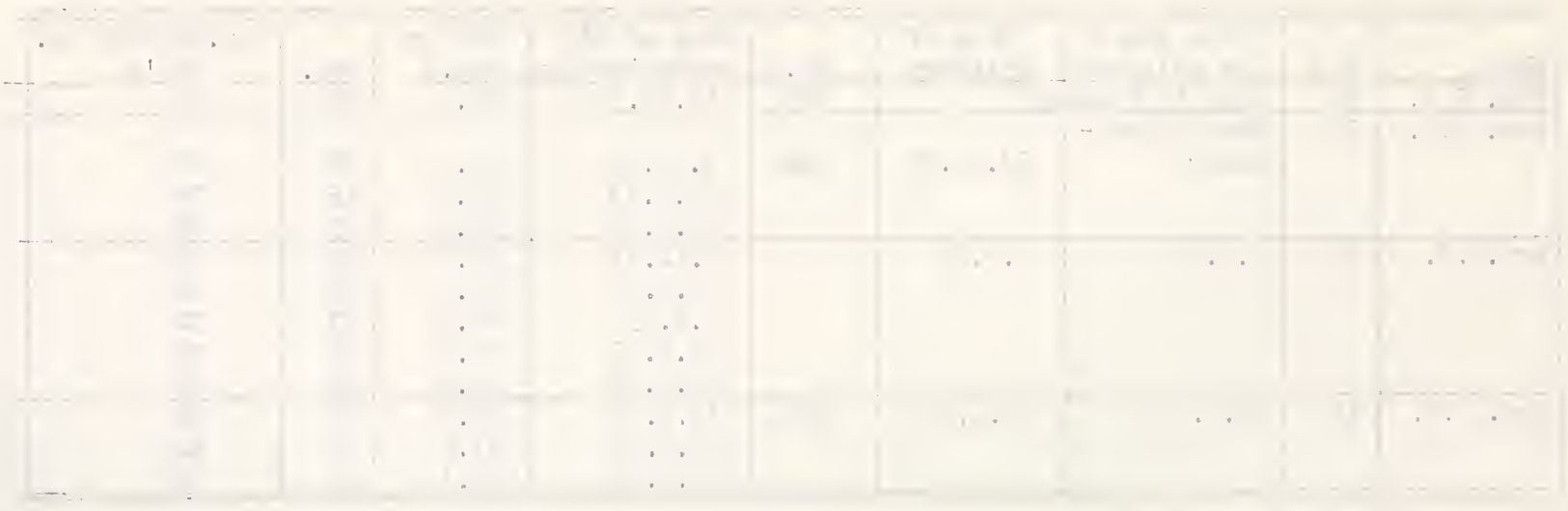
Three cases of pyrexia were studied (see table 27). It will be noted that all glutathione values were within normal limits, except possibly in the case of Miss L.J., who was probably high for her age. It should be noted however, that her hematocrit was low. The glutathione content of erythrocytes in the case of Mr.J.S., taken one month later after he had recovered, showed no appreciable change from when he had a high temperature.

ACTIVE ERYTHROCYTE SEDIMENTATION RATES

Ten patients were studied who had high erythrocytes sedimentation rates (see table 28). The average age was thirty-eight years, and the average glutathione content of erythrocytes was 85 mgms. It should be noted that in only one case was the patient definitely anemic. It is interesting to note that six of these cases belonged to the group of collagen diseases. It would appear therefore that conditions associated with elevated sedimentation rates are also associated often with elevated erythrocyte levels of glutathione.

PREGNANCY

Four cases of pregnancy were studied (see table 29). Of these it was only possible to follow three after delivery. Of the four cases studied, two showed high values of glutathione during pregnancy for their age. It is interesting to note that all three cases followed after delivery showed elevated glutathione values



Name	Age	Date due to be delivered	Date of Delivery	Hg.%	Date of GSH Determination	GSH in mgm.%	Hem.	Mgm.GSH/100 cc. RBC's
Mrs. G.P.	32	Late in February		78%	12.12.52	24.4	38	64
Mrs. R.S.	18	End in December	23.12.52	80%	8.12.52	30.0	38	79
					20.1.53	31.6	45	70
					20.2.53	39.6	45	88
Mrs.A.I.	24	2.2.53	5.2.53		8.12.53	29.7	36	73
					20.1.53	24.0	39	62
					3.2.53	28.8	40	72
					19.2.53	35.0	48	73
					17.3.53	45.0	46	98
Mrs. R.S.	18	28.1.53	23.1.53	82%	15.1.53	32.8	39	84
					27.1.53	33.6	40	84
					26.3.53	40.4	44	92

Table 29 - Glutathione Content of Erythrocytes During and Following Pregnancy.

within two months of delivery. The significance of this finding is not clear. In each case there was an increase in hematocrit following delivery, when it might be expected that glutathione values would fall instead of rising.

MISCELLANEOUS CONDITIONS STUDIED

It was suggested, because of the results found in pulmonary disease, that a rise in glutathione content following lobectomy could be expected. One case was followed (see table 30). Unfortunately the patient developed a haemopneumothorax following left lower lobe lobectomy (for bronchiectasis), and as will be seen developed a marked drop in hematocrit. The changes in glutathione content of erythrocytes however, do vary inversely to the change in hematocrit.

Date	GSH in Mgms.%	Hematocrit	GSH/100cc.packed RBC
20.4.53	25.0	40	63
22.4.53	31.2	31	101
27.4.53	25.2	37	68

Table 30. Glutathione content of Erythrocytes in one case before and after Lobectomy.

One case of renal glycosuria was studied. Blood specimens for glucose taken during the day were within normal limits. The blood glutathione was 33.6 mgms.% with a hematocrit of 47% giving 72 mgms. glutathione /100 cc.packed erythrocytes, which is within normal limits.

Denny-Brown and Porter (247) reported normal blood levels of glutathione in two cases of Wilson's Disease, both before and after receiving B.A.L. (2-3 dimercaptopropanol). One case was followed in this study (see table 32), and he showed consistently low glutathione content of erythrocytes. Liver function tests in this case were all normal. Courses of B.A.L. were given for ten days in November 1952,

and beginning January 6th.1953, and again beginning February 10th. 1953. During the last course, on the third day, he developed a severe reaction and so B.A.L. was stopped and he was restarted on a small dose of B.A.L. on February 16th.1953. There had been clinical improvement in this case with B.A.L. therapy. The association of copper with the oxidation of glutathione (21,46) and the fact that B.A.L. contains two free -SH groups makes this an interesting finding.

date	GSH in Mgms.%	Hem.	Mgm.GSH/100 cc. packed Erythrocytes
21.11.52	23	50	46
6. 1.53	24.4	52	47
13. 1.53	23.6	46	51
15. 1.53	20.8	48	43
10. 2.53	25.2	48	53
17. 2.53	20.4	45	45

Table 31 - Glutathione Levels in a case of Wilson's Disease during BAL Therapy.

One case of hemochromatosis (see appendix) with diabetes, adrenocortical insufficiency and probable liver involvement was studied. The glutathione content of erythrocytes was 47 mgm/100cc. packed erythrocytes which is quite low for a man fifty-eight years of age

One case of delayed puberty, a boy of twelve years (see appendix) showing obesity, hypothyroidism and hypogonadism, had a glutathione content of 62 mgms/100 cc. packed erythrocytes.

One case of non-tropical sprue, a woman aged fifty, had a glutathione content of 72 mgm./100 cc.packed erythrocytes. In this case there was fifteen to twenty pounds weight loss in one year with low serum proteins (total 4.68 gms% with Alb.2.90 and Glob. 1.78).

One case of acute intermittent porphyrinuria, a woman,(see appendix) twenty years old, had a glutathione content of 60 mgms./ 100 cc. packed erythrocytes.

Two cases of gout were studied (see appendix). The first was a man, forty-one years of age, with a serum uric acid of 7.2 mgms.%. His glutathione content was 67 mgms./100 cc. packed erythrocytes. The second case was a man, age seventy-one, with unrecognized diabetes mellitis, whose serum uric acid was 9.7 mgms%. His glutathione content was 76 mgm/100 cc. packed erythrocytes.

One case of hypercholesterolemia, a woman (see appendix) aged thirty-three, with coronary insufficientcy was studied. Her serum cholesterol was 396 mgms.% amd the glutathione content was 68 mgms. /100 cc. packed erythrocytes.

One case of idiopathic hypocalcemia, a woman, (see appendix) age seventy-two showed a glutathione content of 59 mgm/100 cc. packed erythrocytes. It is to be noted that at this time she was quite anemic, (hematocrit 30%), and with her age, one would have expected a much higher glutathione content.

A woman who showed marked improvement on pellets of DOCA implantedm and whose urinary ketosteriods and cortin levels of excretion were within normal limits, showed a glutathione content of 49 mgm./100 cc. packed erythrocytes. Grunert and Phillips (248) found lowered glutathione levels in potassium, chloride, sodiu, and potassium, and sodium chloride defieient rats. They found also that DOCA temporarily reversed the effect of sodium deficiency. They

(249) were also able to show that DOCA exerted some protection against alloxan, and that sodium deficient rats were more susceptible to alloxan. It is interesting that Zwemer et al (250,251) found the potassium tolerance of rats and mice was greatly increased by glutathione, but felt that the glutathione caused a toxic reaction with adrenal cortical stimulation, and that this was the mechanism of protection. It would be of value to study glutathione content of erythrocytes more extensively in patients with marked electrolyte imbalances.

One case of epilepsy, grand mal type, a man aged twenty-seven years showed a glutathione content of 74 mgms./100 cc. erythrocytes, which is at the high limits of normal for his age.

Three cases of atopic dermatitis were studied (see table 32). Two of the three cases showed values within normal limits. The other case, it should be noted had an elevated sedimentation rate which might account for the high value obtained.

Name	Age	Erythrocyte Sed. Rate	GSH in Mgms.%	Hem.	Mgm.GSH/100cc. Packed RBC
Mr.R.S.G.	24	2	29.6	51	58
Mr.M.L.	69	8	32.4	49	66
Mr.L.B.	58	18	38.2	45	85

Table 32 - Glutathione content of Erythrocytes in Patients with Atopic Dermatitis.

DISCUSSION OF RESULTS

1. Normal Values

From the results found, there is a slight but significant increase in glutathione content of erythrocytes with increasing age. This maybe due to decreased efficiency of cardiac and pilmonary function in the older age group. It was difficult to pick what would be considered normal individuals in this age group., especially from the patients in hospital.

2.Conditions Associated with poor oxygen carrying capacity of the blood

In patients with anemia, pulmonary or heart disease, and in the cord blood of newborn babies, an increased glutathione content was found. In all these cases there is a deficiency of oxygen carrying capacity of the blood, either because of a deficiency of hemoglobin, or because of difficulty in the hemoglobin becoming oxidized. The finding of increased affinity of foetal hemoglobin over maternal hemoglobin, and the fact that foetal erythrocytes contain more glutathione than normal erythrocytes, suggests that possibly the elevated glutathione content of erythrocytes in those conditions maybe a factor in oxygen transportation to and from hemoglobin. It is suggested that this would be a worthwhile study at a future date.

3. Nitrgen Mustard Therapy

Nitrgen Mustard therapy does not appear to lower the glutathione content of erythrocytes.

4. Diabetes Mellitis

The glutathione content of erythrocytes in cases of diabetes mellitis does not appear to vaay significantly from the normal values obtained, unless in the case of the severe juvenile diabetics, and a larger series would have to be obtained before one could state there was a significant lowering of glutathione content of erythrocytes in the group. The method of glutathione estimation used is not considered accurate for patients in diabetic acidosis.

5. Anterior Pituitary Gland

ACTH does nor appear to cause a drop in glutathione content of erythrocytes as has been suggested before. The drop in glutathione content of whole blood, as we found, was due to a lowering of the hematocrit, probably due to water retention and a resulting hydremia.

In one case of pituitary insufficiency the glutathione content erythrocytes was found to be within normal limits.

6. Adrenal Cortex

Cortisone does not appear to change the glutathione content of erythrocytes as again, has been suggested before. Hyperfunction of the adrenal cortex, both in Cushing's Syndrome and in the Adrenogenital-Syndrome showed normal values. One case of adrenal cortical insufficiency also showed values within normal limits.

7. Thyroid Gland

In acute thyrotoxicosis there appears to be a lowered glutathione content of erythrocytes, but there did not appear to be any increase in glutathione content during tapazole therapy or following

thyroidectomy.

In hypothyroidism there does not appear to be any marked change in glutathione content of erythrocytes unless there is an associated anemia. Thyroid therapy does not appear to alter the glutathione content of erythrocytes.

8. Liver Disease

Acute infectious hepatitis does not appear to lower the glutathione content of erythrocytes unless in the fatal cases where the patient is in cholemia. In cases of severe hepatic insufficiency, other than of infectious origin, there appear to be a lowering of glutathione content of erythrocytes. The glutathione content of erythrocytes does not appear to have any value as a diagnostic aid in hepatic disease, or in assessing hepatic function.

9. Leukemia

Any worthwhile study of glutathione in relation to leukemia would require a separation of the leucocytes from the erythrocytes which was not done here.

10. Pyrexia

Pyrexia does not appear to alter the glutathione content of erythrocytes.

11. High Erythrocyte Sedimentation Rates

Patients wuth high erythrocyte sedimentation rates appear to have elevated glutathione content of erythrocytes. The significance of this finding is not clear, and might be worth further investigation.

12. Wilson's Disease (Hepatolenticular Degeneration)

One case of Wilson's Disease showed lowered glutathione content of erythrocytes. The association of copper with the oxidation of glutathione (21,46) and the fact that B.A.L. contains two free -SH groups makes this an interesting finding.

13. Abnormalities in Electrolytes

The findings of low glutathione content of erythrocytes in a case of idiopathic hypocalcemia, and one case of DOCA deficiency suggests that a more extensive study of paitients with marked electrolyte imbalances might be worthwhile.

14. Pregnancy

Elevated glutathione content of erythrocytes following pregnancy were found, even with an increased hematocrit, the significance of which is not clear.

SUMMARY

1. The historical background of glutathione, its discovery, the previous basic investigations of its formation and function, and the previous clinical studies are described.

2. The methods for its determination are described.

3. The results obtained from many patients with a variety of diseases are presented.

4. The significance and possible explanation of some of the results observed are discussed.

5. Probable lines of future investigation are indicated.

APPENDIX

BINKLEY ET AL'S METHOD FOR DETERMINATION OF GLUTATHIONE
AS DESCRIBED IN THE JOURNAL OF BIOLOGICAL CHEMISTRY
VOLUME 188, 159 - 162, 1950

Whole blood was collected with heparin as the anticoagulant, (oxalate was found to be unsatisfactory) and a hematocrit was determined; 5 ml. of the whole blood were transferred to an Erlenmeyer flask and 10 ml. of water, saturated with digitonin, were added. After hemolysis was complete 2 ml. of 50 per cent trichloracetic acid were added dropwise with mixing. The flask was stoppered, shaken vigorously, and the mixture was centrifuged until a clear supernatant solution was obtained. 5 ml. portions of the supernatant solution were transferred to each of two colorimeter tubes (calibrated at 7 ml.) and 1 ml. of 2 M phosphoric acid was added to each tube. One tube was heated in a boiling water bath for 90 minutes, and after cooling 1 ml. of 6 N sodium hydroxide was added to each tube. The volume was adjusted to 7 ml. and the method of Sullivan and Hess (9) was applied. The tubes were read (5 minutes after the addition of hydrosulfite) at 500, 540 and 580 mu. in the Coleman junior clinical spectrophotmeter.

Calculation of Results - As has been reported, the reading at 540 is a measure of total cysteine and cysteinylglycine. The ratio between the readings at 500 and 580 mu. is a measure of the relative concentrations of cysteine and cysteinylglycine. The 500:580 ratio for cysteine was found to be 3.4; the ratio for cysteinylglycine was 1.3. The compostion of each was calculated from a graph utilizing these two values. Since all values were expressed as mg. per cent of

glutathione, the standard curve was determined with solutions of glutathione. The values that were obtained were total glutathione (the 540 value of the heated tube), per cent cysteine in the heated tube (from the 500:580 ratio), cysteinylglycine plus cysteine (as glutathione) in the unheated tube, and the per cent cysteine in the unheated tube. From these values the concentration of cysteinylglcine, cysteine, glutathione, and y-glutamylcysteine was calculated. Since no free cysteine was detected in the amount of blood used in these determinations (i.e. the ratio on the unheated tube was 1.3) only the values for cysteinylglcine, y-glutamylcysteine, and glutathione are reported. In practice all values are corrected to a hematocrit of 50 per cent.

METHOD OF SULLIVAN AND HESS (9) FOR DETERMINING CYSTINE IN URINE

To 10 cc. of urine add 4 cc. of 5 per cent NaCN in N NaOH, stir, centrifuge. When ten minutes have elapsed from the time of adding the cyanide, take 7 cc. of the supernatant liquid for colorimetric determination.

To 7 cc. add 2 cc. of 1 per cent 1,2 -Naphthoquinone-4-sodium sulfonate, shake for ten seconds, add 5 cc of 10 per cent anhydrous Na_2SO_3 in 0.5N NaOH, and wait thirty minutes. Then add 2 cc. of 5N NaOH, shake, add 1 cc. of 2 per cent $Na_2S_2O_4$ in 0.5N NaOH.

Match against 5 cc. of an appropriate cystine standard plus 2 cc. of alkaline sodium cyanide and treat as in the above colorimetric work.

MIMEOGRAPHED COPY OF THE METHOD AS RECEIVED FROM DR. BINKLEY

ESTIMATION OF GLUTATHIONE

REAGENTS AND MATERIALS

Heparin Bottles - Add 1.0 cc. of a heparin solution containing 3.0 mg. of heparin per cc, to used penicillin bottles. The bottles are dried in a low temperature vacuum oven, cooled and stoppered with rubber stoppers.

Digitonin Water - 1 gram of digitonin is dissolved in 2 litres of distilled water and the solution is filtered.

50% Trichloracetic acid - 500 cc. of water is added to 500 grams of TCA.

2M H_3PO_4 - 68.5 cc. of 85% H_3PO_4 (14.6M) is diluted with water to a volume of 500.

6 N NaOH - 100 cc. of 50% NaOH (19.0 N) is added slowly to 200 cc. of water.

SULLIVAN -HESS REAGENTS :

5% NaCN in 1 N NaOH - 100 grams NaCN is dissolved in 2 litres of 1 N NaOH (111.1 of 50% NaOH plus warer to make 2 litres).

2% Sodium B-naphthoquinone 4-sulfonate - (made up fresh every day). 20 mgm. of the color reagent /cc. of H_2O.

10% Na_2SO_3 in 0.5N NaOH - 200 grams of Na_2SO_3 dissolved in 2 litres of 0.5n NaOH (55.5 cc, 50% NaOH added to water to a volume of 2 litres)

1% Na_2SO_4 in).5N NaOH -(made up fresh just before use). 10 mgm. $Na_2S_2O_4$ per cc. of 0.5N NaOH (27.7 cc 50% NaOH added to water to a volume of 1 litre).

PROCEDURE :

The heparinized blood is well mixed and 5 cc. is drawn off accurately with a volumetric pipet into a 50 cc. Erlenmyer flask. 10 cc. of digitonin water is added and the solution is well mixed and allowed ti stand for five minutes. 2 cc. of 50% TCA is added, the flask immediatly stoppered with a rubber stopper and the contents mixed vigorously. After mixing well, the contents of the flask are transferred to a centrifuge tube and centrifuged until a clear filtrate is obtained. To obtain a clear filtrate free from precipitate, the filtrate is generally transferred to a second tube and centrifuged again. Two 5 ml samples of the clear filtrate are transferred to colorimetric tubes calibrated to 7 cc. 1 ml. of 2M H_3 PO is added to each of the tubes and the solutions are well mixed. One tube is placed in a boiling water bath for 90 minutes. After heating, the tube is cooled by immersion in cold water. 1 ml. of 6 ON NaOH is then added to each tube. The volume in the heated tube is corrected to 7.0 with water.

SULLIVAN-HESS DETERMINATION

2 cc. of 5% NaCN in 1 N NaOH is added to all the tubes. The tubes are shaken and allowed to stand for ten minuted. Then 1 cc. of the color reagent is added to the first tube and the tube is shaken vigorously for 30 seconds. 1 cc. of the color reagent is then added to the second tube which is then similarly shaken. Then 5 cc, of 10% Na_2 SO_3 in 0.5N NaOH is added to the first tube and the contents are

thoroughly mixed by shaking the tube five times. Color reagent is then added to the third tube and shaken thirty times. 5 cc. of 10% Na_2SO_3 is then added to the second tube and so on ..... (when shaking the tubes, the tubes are flipped from the wrist),

The tubes are allowed to stand for thirty minutes. When more than 10 or 11 tubes are involved, the Sullivan test is run on a staggered series of no more than 10 tubes in one series.

The tubes are well shaken and 2 cc. of 1% Soldium hydrosulfite in 0.5N NaOH is added to each. The tubes are again well shaken and are read in the colorimeter 5 minutes after the hydrosulfite has been added. It has been found that when the tubes have been well shaken throughout the entire procedure, the colorimeter readings remain constant for about twenty minutes after the addition of Na hydrosulfite.

o/o Transmission of the tubes is read at 540 mu. The optical density of the heated tubes are read at 500 and 580 mu.

A blank tube is run with every series and contains : 2cc. H_2O, 2.5 cc. digitonin H_2O, 0.5 cc.50% TCA, 1,0 cc. 2 M H_3PO_4 1.0 cc. 6 N NaOH.

GLUTATHIONE STANDARDS:

A solution containing 1 mg. GSH/cc. or 0.5 mg.GSH/cc. is made up in a volumetric flask. In the usual series, one will have a blank tube and tubes containing 0.2 mg., 0.75 mg. and 1.0 mg.GSH. The volume in all the tubes is made up to 2cc. with water. 2.5 cc. of digitonin solution is added to each tube followed by 0.5 cc. 50% TCA and 1.0 cc. of 2M H_3PO_4. The tubes are shaken after the addition of each reagent and all the tubes with the exception of the blank are put in a boiling

water bath for ninety minutes. As outlined in the procedure the tubes are cooled, neutralized with 6N NaOH and made up to a volume of 7.0cc. with water. A Sullivan-Hess test is run. Colorimetric readings of the tubes are made at 540 mu. It has been found that 0.5 mg. GSH will give a reading of from 53 to 56%.

A standard chart is set up such that mgm. o/o GSH reading may be made directly.

A correction for the dilution of blood is made as follows :

$$\frac{\text{5cc. blood used}}{\text{17 cc.total filtrate}} = 0.294 \text{ cc. blood/cc}$$

$$5 \text{ cc.} \times 0.294 \text{ cc.blood/cc} = 1.47 \text{ ml. blood /5 cc. filtrate}$$

$$x \text{ mgm. GSH} \times \frac{100}{1.47} = \text{mgm. o/o GSH}$$

The mgm. o/o GSH is plotted against o/o Transmission on a 1 cycle semi-log graph paper.

Cysteinylgylcine is read in the unheated tube at 540 mu.

GSH is read in the heated tube minus the reading of the cysteinylglycine.

A total GSH is that reading obtained in the heated tube alone.

The percentage of glutamylcysteine is obtained from the ratio of the density at 500 to that at 580. The 500/580 ratio for cysteine and for hydrolyzed glutathione (cysteinylglcine) should be determined for the individual colorimeter. Our values have been found to be 1.3 for cysteine and 3.6 for cysteinylglcine. Per cent cysteine (or glutamylcysteine) is a straight line relationship so that at a ratio of 1.3 the percentage is zero and at 3.6 the percentage is 100.

THE NITROPRUSSIDE METHOD FOR THE DETERMINATION OF BLOOD GLUTATHIONE

by

Thompson, R.H.S. and Watson,D., J. Clin Path.5, 25 -29, 1952

Reagents

(1). 10% (W/V) trichloracetis acid (AR).

(2). Ammonium sulphate, solid (AR).

(3). Ammonium sulphate, saturated aquesous solution.

(4). M-sodium nitroprusside (AR), 0.05 in saturated ammonium sulphate.(AR)

(5). 8N Ammonium hydroxide (AR)

(6). Standard glutathione (B.D.H.) solution 1 mg./ml. This solution must be freshly prepared for each estimation.

Method

0.5 ml of freshly drawn blood (oxalated or heparinized) is added to 1.5 ml. of glass distilled water in a 15 ml. centrifuge tube, and allowed tostand for five minutes to lake. Then 2 ml. of 10% trichloracetic acid are added, and the mixture well stirred with a glass rod and allowed to stand for five minutes, after which it is centrifuged for five minutes, at 3,000 r.p.m. Then 2 ml. of the clear protein-fresh supernatant are pipetted into a stoppered 10 ml. graduated cylinder. Solid ammonium sulphate is added to saturate the solution (by tipping in 1.4 g. contained in a marked tube); after shaking the volume is adjusted up to 3 ml. with saturated ammonium sulphate.

For the production of color 0.5 ml. of 0.05 M-nitroprusside reagent is added, followed by 0.7 ml. of 8N ammonium hydroxide.

After transferring into cells of 1 cm. optical depth the color

intensity is read in a photoelectric colorimeter 30 seconds after the addition of the ammonium hydroxide, using an Ilford spectrum green (604) filter. A reagent blank determination is carried out using 1 ml. water and 1 ml.10% thrichloracetic acid, with all subsequent additions as for the blood filtrate. All tubes are compared against water.

Standards

The standard glutathione solution is diluted tenfold to give a solution containing 100 ug GSH/ml. To prepare a standard curue 0.2, 0.4.....1.0 ml. samples (i.e.20,40,. .100 ug. GSH) are each diluted with water to 1.0 ml. The 1.0 ml. of 10% trichloracetic acid is added to each, and the estimation carried out as for the blood filtrates. One standard tube (containing 60 or 80 ug.GSH) is included in each series of blood estimations.

Normal Glutathione Content of Erythrocytes

	Age	Condition	GSH in mgm.%	Hem.	Mgm.GSH/100 cc. Packed RBC
1.	5	Poliomyelitis recovering	21.6	38	57
2.	8	Poliomyelitis recovering	28.8	43	67
3.	8	Poliomyelitis recovering	28.4	42	68
4.	8	Poliomyelitis recovering	31.6	48	66
5.	12	Osteogenesis Imperfecta	26.6	45	59
6.	12	Healed burns	26.3	38	69
7.	12	Poliomyelitis, recovering	24.8	45	55
8.	12	Poliomyelitis, recovering	24.2	41	59
9.	14	Poliomyelitis, recovering	24.4	46	53
10.	16	Congenital Hip	30.6	45	68
11.	17	Poliomyelitis, recovering	29.0	53	55
12.	19	Poliomyelitis, recovering	24.4	41	60
13.	19	Poliomyelitis, recovering	24.4	46	53
14.	19	Poliomyelitis, recovering	25.5	50	51
15.	21	Poliomyelitis, recovering	27.0	46	59
16.	21	Poliomyelitis, recovering	28.8	53	54
17.	21	Poliomyelitis, recovering	28.2	44	64
18.	22	Previous Fractures	35.4	52	68
19.	23	Poliomyelitis	26	41	63
20.	23	None	30.4	49	62
21.	26	None	36.8	52	71
22.	27	Injured Rectum	33.1	49	68
23.	28	Poliomyelitis, recovering	28.0	44	64
24.	29	Poliomyelitis, recovering	26.1	45	58
25.	30	Poliomyelitis, recovering	24.6	47	52
26.	32	Poliomyelitis, recovering	33	43	77
27.	32	None	27.6	46	60
28.	35	Poliomyelitis, recovering	22.8	44	52
29.	37	Poliomyelitis, recovering	31.5	45	70
30.	39	Poliomyelitis, recovering	29.8	42	71
31.	41	Back Strain	30.0	44	67
32.	44	Spinal Fusion	34.4	47	73
33.	47	Healed Fracture	30.2	46	66
34.	47	Meniscectomy	29.6	39	76
35.	47	Intravertebral Disc	36.8	45	81
36.	50	Healed Fracture	30	53	57
37.	53	Healed Fracture	32.8	49	67
38.	57	Painful back	24.0	39	62
39.	57	Osteoarthritis	24.8	44	56
40.	63	Injured Hip	30.4	49	62
41.	63	Previous Jundet Operation	23.2	37	63
42.	63	Osteoarthritis	30.5	50	61
43.	64	Osteoarthritis	32.8	50	66
44.	68	None	35.2	46	77
45.	68	Healed fractures	29.5	44	67
46.	69	Osteoarthritis	33.2	44	76
47.	69	Osteoarthritis	34.3	52	66
48.	72	Healed Fracture	26.8	41	65
49.	73	Osteoarthritis	21.6	36	60
50.	77	Healed Fractures	33.2	50	66
51.	80	Healed Fractures	38	53	72

APPENDICES

Glutathione Content of Erythrocytes in Conditions Not Shown in the Tables

Disease	Name	Age	GSH in Mgm.%	Hem.	Mgm. GSH/100 cc. RBC	Comments
Adrenal Cortical Insufficiency.	Mrs.A.J.	23	29.2	43	68	
Hemochromatosis	Mr.N.S.	58	21.6	46	47	Associated Diabetes Mellitis, Adrenal Cortical Insufficiency, and probably some liver involvement.
Delayed Puberty	Mr. N.O.	12	31.2	50	62	High pitched voice. Genitalia undeveloped. BMR -16 -21.
Acute Intermittent Porphyrinuria	Mrs. C.C.	20	27	45	60	
Non-Tropical Sprue	Mrs. H.W.	50	32.4	45	72	Marked weight loss. Low serum protein. (Total 4.68 gm.%).
Gout	Mr.R.B.	71	34.4	45	76	Unrecognized Diabetes. Serum Uric Acid 9.7 mgm.%
Gout	Mr.M.W.	41	24	36	67	Serum Uric Acid 7.2.mgm.%
Hypercholesterolomia	Mrs. D.M.	33	27	40	68	Serum Cholesterol 396 mgm.% BMR -13 -18.
Idiopathic Hypocalcemia	Mrs.V.A.	72	17.8	30	59	Also suffering from anemia.
Possible DOCA Deficiency	Mr. M.B.	39	21	42	49	
Epilepsy /Grand Mal	Mr. M.D.	27	34.6	47	74	
Hypopituitarism	Mrs.W.S.	58	32.1	45	71	Chromophobe Adenoma of the Pituitary.

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